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**PROCEEDINGS
OF THE
CONFERENCE
ON
ATMOSPHERIC CONTAMINATION
IN
CONFINED SPACES**

30 MARCH - 1 APRIL 1965

DECEMBER 1965

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AEROSPACE MEDICAL RESEARCH LABORATORIES
AEROSPACE MEDICAL DIVISION
AIR FORCE SYSTEMS COMMAND
WRIGHT-PATTERSON AIR FORCE BASE, OHIO

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FOREWORD

The Conference on Atmospheric Contamination in Confined Spaces was held in Dayton, Ohio on 30, 31 March and 1 April 1965. Sponsor was the Aerospace Medical Research Laboratories, Aerospace Medical Division, Air Force Systems Command. Arrangements were made by the Toxic Hazards Research Unit of Aerojet-General Corporation under the terms of Contract AF 33(657)-11305. The Toxic Hazards Research Unit is located at the Toxic Hazards Branch, Physiology Division, Biomedical Laboratory at Wright-Patterson Air Force Base, Ohio. Dr. Anthony A. Thomas, Chief, Toxic Hazards Branch, and Dr. George Kitzes, Chief, Physiology Division, served as Co-Chairmen.

Acknowledgment is made on behalf of the Aerospace Medical Research Laboratories to Colonel Frank Townsend, Aerospace Medical Division, Brooks Air Force Base, Texas, for his introductory remarks and support, to the session Chairmen, the many speakers, panel members and their respective organizations for their response and cooperation. Special thanks are due Captain John J. Jurgiel, 1/Lt. Duncan McVean and M/Sgt. John Naylor of the Toxic Hazards Branch, and to Mr. Paul E. Cowdin of Aerojet-General Corporation, for their efforts in coordinating the countless details which contributed so much to the successful completion of this Conference.

ABSTRACT

This report is a complete compilation of the papers presented and the proceedings of the Conference on Atmospheric Contamination in Confined Spaces, sponsored by the Aerospace Medical Research Laboratories and held in Dayton, Ohio on 30, 31 March and 1 April 1965. Major technical areas discussed by the invited speakers, panel members and Conference attendees included continuous inhalation exposure techniques, statistical methods for evaluation and interpretation of exposure data, minimum criteria for continuous exposure studies and toxicological qualification of space cabin materials. The Conference participants were provided the opportunity to tour the Toxic Hazards Research Unit at Wright-Patterson Air Force Base and to ask questions regarding its operation. A discussion of the Clean Air Act of 1963 was presented by a representative of the Federal Department of Health, Education and Welfare.

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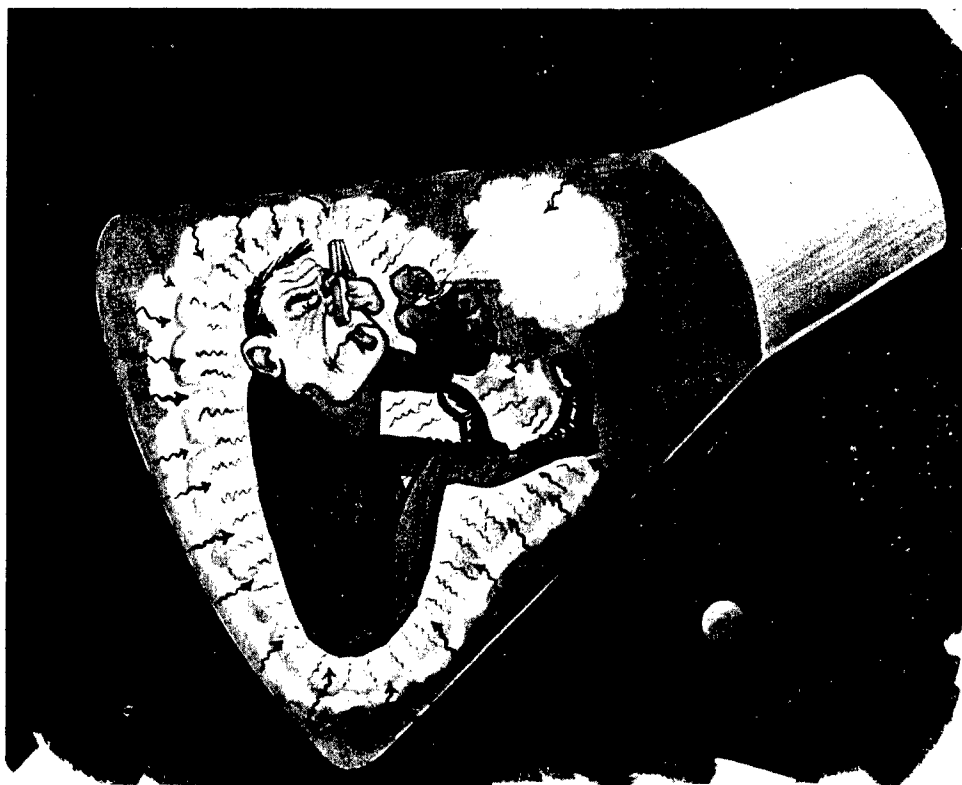
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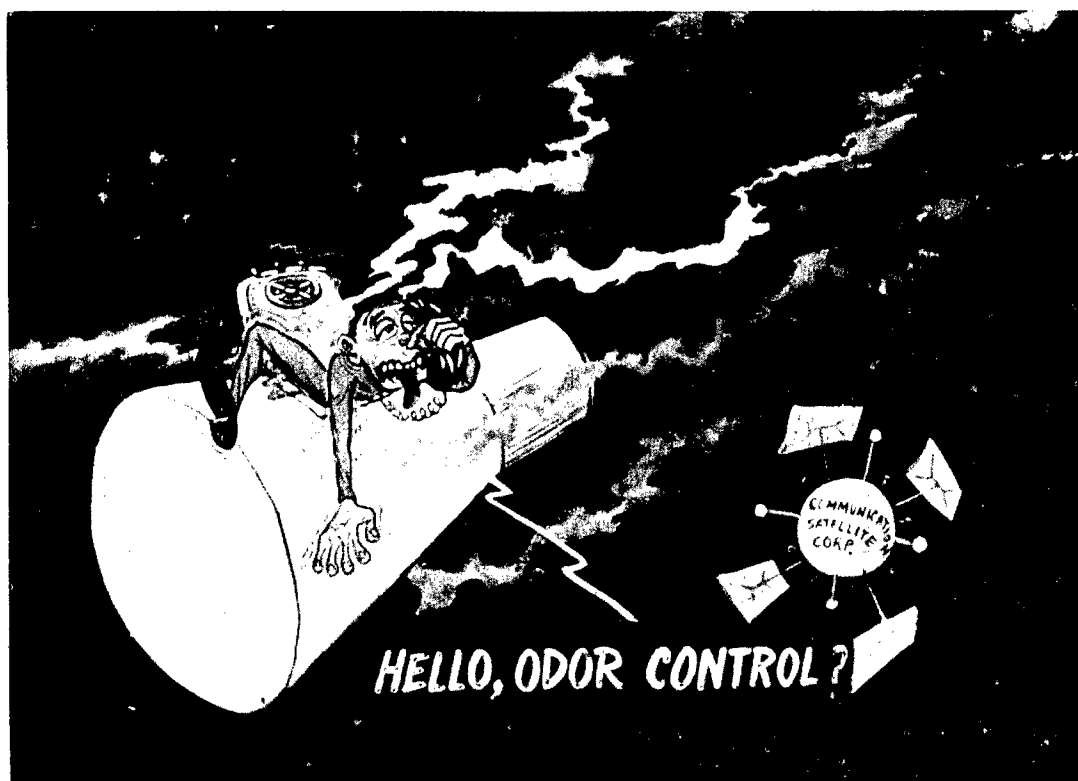
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CONTAMINANT SOURCES



HELLO, ODOR CONTROL?

POSSIBLE CONTAMINANT EFFECT

Frontispiece

INTRODUCTION

The problem of atmospheric contaminants in sealed cabins and confined spaces is not new and was recognized during World War II in connection with submarines and overcrowded air raid shelters. While at that time it was more of a nuisance than a real health hazard, with the advent of prolonged submergence in nuclear submarines and the rapidly extending mission duration of manned space exploration, it now becomes a matter of not only survival but also preservation of performance and prevention of serious disability. Two earlier symposia have dealt with some aspects of toxicity in the closed system. The first of these was conducted in 1958 in New London, Connecticut, under the title "Symposium on Submarine and Space Medicine", and was sponsored by the Advanced Research Projects Agency in cooperation with the American Institute of Biological Sciences. The United States Naval Medical Laboratory was the host. The latter symposium held one session on toxicological problems in confined spaces. The second symposium, titled "Toxicity in the Closed Ecological System", was held in July 1963 in Palo Alto, California, under the sponsorship of the United States Navy Special Projects Office and the Lockheed Missiles and Space Company. Topics for the sessions included origin and effect of contaminants, evaluation of toxicity, detection of contaminants, monitoring and control methods.

As our research methods and techniques in toxicology progressed to the point where truly uninterrupted, long-term continuous exposures became feasible, many new technical difficulties were discovered, and the costliness of this work became very apparent. The need for a scientific meeting dedicated primarily to research problems in toxicology became increasingly urgent. Because the number of facilities available to conduct such work is limited in the free world, it has also become critically important that researchers in these facilities should produce data that are directly comparable. This would prevent unnecessary or costly duplication of experiments and loss of valuable biological lead time. Although everyone abhors "standardization" in research and finds it stifling of initiative, inevitable methodology must be standardized in the sense that only acceptable methods that can furnish reproducible and meaningful information should be used. Thus, the setting of minimum criteria for research methodology becomes, in fact, a sort of quality control procedure, a highly desirable situation in any research endeavor.

In view of the urgent need for exchange of ideas among the leading scientists in this field and some agreement on basic experimental design, with strong encouragement from Colonel Alvin Meyer of the Surgeon General's Office, Headquarters USAF, and Dr. Harry Hays of the Advisory Center on Toxicology, National Research Council, National Academy of Sciences, this Conference on Atmospheric Contamination in Confined Spaces was conceived and organized.

Obviously, this meeting was not intended in any way to become a symposium. The issues at hand required a very practical approach to many matters of technical detail, and the guiding thought was one of trade-off between optimum experimental design and minimum requirements for comparability of data. To provide timely and useful toxicological information to the design engineers one must also compromise some of the goals which the ultimate perfectionist seeks. This compromise then is necessitated by two crucial and overriding factors, available time and money. Therefore, we felt that a conference which provided a workshop atmosphere was much more desirable than would be the highly academic approach of a formal symposium.

We also knew that this Conference could not answer all the questions which must arise but hoped that it would be the first step toward the development of close individual relationships among the workers in this field, and that some mutual agreement on desirable and undesirable methodology might be reached. Therefore, we plan to follow up on an annual basis, and as we learn from each other the past years' failures and frustrations may lead to next year's success.

We cannot run unless we first learn to walk. If the approach of this year's Conference program has divulged an air of naïvete by asking such obvious questions as preferable methodology, minimum number of animals to be used, acceptable exposure chamber techniques, etc., the proceedings, the papers and the discussions perhaps justified this unsophisticated approach. Some points were raised where agreement could not be reached, and the discussion of many seemingly simple parameters has revealed that there is more to these than what meets the eye. We have mutually benefited from our panel discussions and questions from our Conferees.

We sincerely hope as experience grows and as research data accumulate that there will be a gradual tendency in this series of conferences toward the makings of a real symposium. Meanwhile, I must take the blame for arranging the technical program for this very first Conference.

Dr. Anthony A. Thomas

WELCOMING REMARKS

By

Jos. M. Quashnock, Colonel, USAF, MC

Commander
Aerospace Medical Research Laboratories

The United States Air Force is now engaged in a scientific program to determine the toxic hazards that are generated in the atmosphere of aerospace systems and to develop tolerance limits to atmospheric contaminants for various mission durations.

Research supporting this program is scattered over many government and industrial laboratories, and is carried out by a large number of independent investigators, using a wide variety of approaches and an even wider variety of methodology. This work, by its very nature, is time consuming and expensive. There has been a considerable body of data collected which has not been published, and in the process of doing this research, valuable experience has been gained by every investigator which can benefit the research community as a whole.

There are three major problem areas to which this Conference should address itself:

1. The maintenance of truly uninterrupted, long term continuous exposure, coupled with the simulation of environmental parameters, represent serious design problems in equipment and have increased the already expensive costs of inhalation exposure studies.
2. Statistical evaluation, although not radically different for continuous exposure work, does pose certain problems in trade-offs between the number of animals used per dose level and the cost of exposure, the evaluation of subtle trends and the treatment of the control group. In the area of interpretation, the pitfalls and the difficulties of extrapolation of animal data to human tolerance is a very sensitive area.
3. Selection and the so-called "toxicological" methods in qualifying space cabin materials are widely divergent and are in a state of complete chaos. In their complexity, they have varied from a simple "sniff test" to full scale analytical chemistry efforts, including highly sophisticated instrumentation. Minimum requirements for the study of "gas-off" products should be considered, so that comparable and meaningful data can be compiled throughout the nation's aerospace industry and the governmental laboratories.

Thus, the very purpose of this Conference is to identify the scientifically feasible and economically sound trade-offs in continuous inhalation exposure techniques and analytical procedures that ultimately are needed for the toxicological acceptance of cabin materials and atmospheres.

We sincerely hope that this Conference will be the first important step toward the solution of these problem areas. Your attendance and participation in these meetings is an important contribution to this Conference. We hope to maintain a workshop atmosphere and a free exchange of ideas. We look forward to your active participation.

In closing, allow me to quote Harvey Cushing, who has so beautifully expressed the true need for personal communication and gatherings among scientists in his book titled "The Life of Sir William Osler":

"By no means the smallest advantage of our meetings is the promotion of harmony and good-fellowship. Medical men, particularly in smaller places, live too much apart and do not see enough of each other. In large cities we rub each other's angles down and carom off each other without feeling the shock very much, but it is an unfortunate circumstance that in many towns, the friction being a small surface, hurts and mutual misunderstandings arise to the destruction of all harmony. As a result of this may come a professional isolation with a corroding influence of a most disastrous nature, converting a genial, good fellow in a few years into a bitter old Timon, railing against the practice of medicine in general and his colleagues in particular. As a preventive of such a malady, attendance upon our annual gatherings is absolute, as a cure it is specific. But I need not dwell on this point -- he must indeed be a stranger in such meetings as ours who has not felt the glow of sympathy and affection as the hand of a brother worker has been grasped in kindly fellowship."

INTRODUCTORY REMARKS

By

Frank Townsend, Colonel, USAF, MC

Vice-Commander
Aerospace Medical Division

General Bedwell hoped that he could be here but our schedule has been so tight in the last few weeks that it was impossible for him to make it. However, I always have the good fortune of being the backup man in that system and I'm very happy to be here and to bring you greetings from the Commander and the staff of the Aerospace Medical Division. As you know, in the Division, the Aerospace Medical Laboratory here at Wright-Patterson is our lead laboratory in the study of toxicology, and problems relating to toxicology. The School of Aerospace Medicine at Brooks and the Aeromedical Laboratory at Holloman both are participating actively in this program with Dr. Thomas and his staff. I think this Conference becomes very timely because we have arrived at what may be really a crossroads. We have got to make some decisions in the field of toxicology. We may regard where we are if we proceed to look at what has gone on in the past, try to evaluate where we are today, and, hopefully, make wise decisions for the future. The present day industrial toxicology standards have evolved over many years. The techniques of testing in both occupational and industrial situations could evolve together. The tempo, although it may have seemed fast to us in the past, was nothing as compared to the tempo we are faced with today. So we did have time. In most of these situations man was exposed, within a limited time frame. Today we are confronted by a true "revolution", if you will use the term, in the development of new materials, used in every imaginable way; paint, coatings, wiring, insulation, and so on, throughout all of the environmental systems in which man must operate in the present day technological world, whether it be in long-range aircraft, in space craft, programmed not for one or two days, but programmed for many days or even months, in wind tunnels, simulators and all of the other systems and support activities which go to make up the complex technological basis of our system evolvement. So, we no longer can think of the 8-hour day, as Colonel Quashnock has pointed out, and we no longer can think of the five or six day a week standard. And, as he has so aptly noted, we need more uniform and exact methods of measuring these products. This is a tremendous job, and in the military and in our Division, we are faced with development implementing standards for these new materials. What will be the result of aging of some of these relatively new products? We've only had a limited time to study them. - All this adds up to where we are, and we are truly at a crossroads. I think that we can look forward to this meeting being a very signal one in giving us guidance as to which is the path to the future. We have many critical decisions to make. We must have

the proper information to permit these decisions to be based on facts rather than on extrapolations or guesswork. We, all of us, have a vital stake in this problem. Our nation has a vital stake in it, whether we are here from the military, from other Government services, from universities, from industry; regardless of where we come from, we have in common the problem that we're going to be discussing the next couple of days.

So, I'm looking forward very much to the papers that are to be presented at this Conference and the discussion that evolves. We must realize that the magnitude of this problem is going to take the ingenuity of all of us to accomplish.

SESSION I

CONTINUOUS INHALATION EXPOSURE TECHNIQUES

Chairman

Mr. Verald K. Rowe
Biochemical Research Laboratory
The Dow Chemical Company
Midland, Michigan

SESSION I

Opening Remarks Mr. V. K. Rowe, Chairman

In industrial toxicology studies we are seldom confronted with exposures lasting more than 8 hours and rarely with exposures lasting 12 to 16 hours. However, when we start to think of the problems of air pollution or life in confined spaces, we must think in entirely different terms.

In exposures lasting 8 hours, or less than 24 hours, per day, there is some time in each day when the subject, or the biologic medium, has time to recover, time to eliminate the material or materials to which he has been exposed; but, in truly continuous exposures, no such time is available. Consequently, the tolerable dose or exposure must not exceed that which the organism can excrete or tolerate without adverse effects for an indefinite period of time.

The determination of such doses, the means of maintaining such and the evaluation of the effects are problems to which this Conference is dedicated.

The generation and maintenance of truly uninterrupted, long term continuous exposures, coupled with the simulation of environmental parameters that may be expected for certain missions, represent serious design problems in equipment.

The determination of intensity of the exposure requires rather sophisticated analytical equipment.

The parameters used in the selection of appropriate animals or even human subjects for such studies and the measurement of effects, if any, also require careful observation and evaluation.

The purpose of this session is to discuss reasonable approaches to the overall area of continuous exposure techniques and to suggest minimum criteria which, if met by all investigators, will permit comparison of data from one laboratory to another and will prevent the necessity for repeating this expensive work.

CHAMBER EQUIPMENT DESIGN CONSIDERATIONS FOR ALTITUDE EXPOSURES

By

Anthony A. Thomas, M.D.

Toxic Hazards Branch
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Wright-Patterson Air Force Base, Ohio

The exposure chamber design for performing inhalation studies for industrial toxicology purposes is well-defined. The equipment must be capable of controlling certain environmental parameters (Table I) so that the undesirable variables from one experiment to another can be eliminated and the pertinent variables can be uniformly maintained throughout the study.

Unfortunately, a great number of the variables are biological and lend themselves only to a limited amount of standardization, most of which can be effected only by careful planning of the experimental procedure, selection of animal species, and adequate clinical laboratory studies to assure acceptable uniformity of the exposed population.

Popular chamber designs in general are not suited for altitude exposure work without either extensive modification or severe restrictions in functional capability (Table II). As our criteria increase from bare minimums to optimum simulation of the atmospheric parameters in space cabins and the type of exposure experience, the costs increase proportionally because of structural design requirements for altitude work and for the necessary extent of automation for continuous, around-the-clock, exposure work.

Fortuitously, the chamber volume per se does not considerably reduce the total cost but does considerably affect the versatility of equipment. (Table III) Moreover, in opposition to short term exposure work, rapid chamber equilibration of contaminant concentration and other atmospheric parameters becomes unimportant if continuous exposure schedules, lasting from several days to months, are the final objective.

A typical inhalation exposure unit for industrial toxicology studies is depicted as a flow diagram on Figure 1. Although only temperature, humidity, air flow rate and contaminant concentrations are controlled here, the complexity of equipment is impressive and, if some automation is required, the price tag per chamber unit can vary from \$10,000 - \$60,000.

The altitude exposure unit now in use at our Laboratory is illustrated in flow diagram fashion in Figure 2. Although it does require the same basic components as the industrial type exposure unit, some of these have been omitted for the sake of clarity. The obvious basic differences are related to the additional equipment that is required to maintain an exotic environment. Oxygen (liquid or gaseous) must be stored and supplied to the chamber, vacuum pumps must be provided to maintain the necessary pressure differential, the exposure chamber must be sturdy enough to tolerate at least 10 psi pressure differential, with a safety factor of 4 (40 psi total), it must be leak-proof so as to prevent undue dilution of the oxygen concentration by outside air, and, finally, to permit uninterrupted exposure of long duration, and to permit the feeding and care of animals, an airlock system must be provided. Moreover, the automation requirements are much heavier with this kind of equipment, and sufficient redundancy of crucial equipment must be provided to prevent the loss of a long term experiment near its completion due to equipment breakdown. This would be intolerable, from the standpoint of manpower, economy and time investment. The salient characteristics of this exposure unit will be explained in more detail a little later in this paper. You will also have a chance to tour our facility.

Returning to the question of chamber size, for screening of space cabin materials and their mixtures, small chambers are desirable because of the scarcity of some of the material and the striving to simulate and "scale" the quantity and proportion relations existing in the space cabin. The flow diagram shown in Figure 3 is even more simplified than the previous ones because it does not show the flow diagram of the environmental envelope that is required to maintain the small animal exposure chamber within the 5 psia, 100% oxygen atmospheric parameter. The most obvious new additions of equipment are the gas-off oven to heat space cabin materials to their working temperatures and the addition of a life support system to scrub out carbon dioxide and replenish oxygen in the recirculating contaminant/oxygen stream. Recirculation is absolutely necessary to permit buildup of contaminants and gas-off products in a closed system such as a spacecraft.

Description of the Thomas-Dome Facility

The effects of truly uninterrupted, prolonged and continuous exposure to contaminants in the atmospheres of sealed cabins are extremely deleterious even at normal atmospheric pressure and composition. With certain contaminants, concentrations not exceeding their Industrial Threshold Limits have proven fatal to animals in the course of 90-day continuous exposure. Since most space systems operate at reduced atmospheric pressures and oxygen rich environments, it became necessary to study the true impact of such environments upon the resistance of the organism to specific and non-specific chemical stress and to obtain basic information on the conceivable potentiation or mitigation of toxic effect by the unusual environmental. A radically new facility was designed and is operating at the Aerospace Medical

Research Laboratories, W-PAFB, Ohio, to perform inhalation studies with space cabin materials. These dome-shaped exposure chambers (Figure 4), 9 feet tall, 12 feet in diameter, provide complete visibility of the test subjects, altitude capability to 28,000 feet (approximately 5 psi or 1/3 atmosphere), and uninterrupted exposure by means of a vertical airlock system.

The top portion of the domes can be separated from the bottom to facilitate loading of cages and animals or life support equipment. Air-tight seal is achieved by an O-ring pressurized to 40 psi.

The operation is automated to the point where a single person can care for the facility during off-duty hours. The Minneapolis-Honeywell Recorder controllers on the panel control oxygen-CO₂ concentration, oxygen flow rate, temperature, contaminant flow, absolute pressure and dew point (R. H.), within each dome independently.

Black floor, walls and ceiling facilitate both photographic observation and sensory deprivation. Other features are the diffuser for the oxygen-air-contaminant mixtures, and the peripheral exhaust duct. Both of these and the dome configuration are crucial in obtaining good uniformity of contaminant concentration at any level.

Liquid oxygen storage is of 68,000 pounds capacity. A converter-heater supplies gaseous oxygen at 50 psi pressure and 72° F to the chambers. The container is kept full to prevent concentration of contaminants in the oxygen supplied to the facility.

A high pressure blower supplies filtered and preconditioned outside air which is used instead of gaseous nitrogen for making up various oxygen-nitrogen mixtures at higher than 5 psi atmospheric pressure environments. Correction is made for the oxygen content of the air.

Vacuum pumps are provided in triplicate to prevent loss of an experiment due to equipment breakdown. Other vital types of equipment that are redundant are airconditioning, oxygen heaters, air compressors and electrical power (150 KW diesel generator).

The sewage and waste disposal tank is located on a lower level and can be pumped down to a slightly lesser pressure than the domes. Analytical instrumentation is conveniently located for verifying the contaminant stream concentration prior to mixing with the oxygen flow.

All controls are pneumatically operated, and the compressor equipment is redundant. Three packaged chillers are also used.

Pneumatic control piping is color-coded for easy trouble shooting. Red for the main air supply, 100 pounds pressure; orange for the control

signal for valves, 3 to 15 pound; yellow for the feedback from recorder controllers on the console; and violet for the solenoid signals.

Valves regulate the rate of flow between 10 to 100 cfm for each dome, automatically and independently. Oxygen or oxygen/air mixtures are hard set at the beginning of the experiments for each dome.

The dome penetration plate has the following connections: externally operated water flush system and drinking water for dogs; sampling port; connections to the oxygen sensor; hard line communication; 24 pairs of shielded cable for biological signals; pressure sensor (large read-out dial and console recorder/controller).

We have both visual and audio alarm capability for adjustable tolerances in ρO_2 , ρCO_2 , oxygen and contaminant flow rate, pressure, temperature and humidity.

Contaminant generating systems are also in the basement. The contaminant is metered into the preconditioned oxygen or oxygen-air stream in the proper proportion. Flow rate of the contaminant is hard set, but is continuously monitored by a console. Either continuous monitoring or three wet chemical analyses per day assure a not more than 10% deviation of actual to nominal contaminant levels.

Shatterproof, one-inch thick glass paneling permits unobstructed view of all animals, as well as excellent time-lapse photographic record of spontaneous activity in the dog pens. The vacuum safety valve is set at the proper level to guard against runaway pumping. A three-inch dump valve, redundant for inside and outside operation, permits rapid repressurization in emergency situations.

Dome window retaining clips are spring-loaded. Double neoprene gasketing keeps the leak rate below 0.5 lb. per 4 hours in a static condition with 10 psi differential. This permits 98% oxygen content at 5 psi dynamic flow of only 18 cfm oxygen.

Safety features include such things as a dome hook-lift portion which must be engaged into the overhead crane before entry into the dome is made. In case of fire, the dome is repressurized in 15 seconds by the dump valve. Two outside observers must be present during all entries. The dome is lifted by the crane as soon as repressurization allows and supporting pillars are inserted.

In addition, automatic sprinklers and deluge heads can also be manually operated.

Monkey cages can hold 6 monkeys. Other items of interest are the loudspeaker alarm system, the open airlock with protective railing, the circular water spray ring and the water hose for cleaning.

Dog pens for males and females hold a total of 10 to 12 animals. A fold-down utility table facilitates blood drawing.

The cable box has 24 shielded, double-pronged Cannon plugs, and a lint filter on the circular exhaust duct also houses the polarographic oxygen sensor.

A vertical airlock is provided which has an emergency dump valve, observation port hole and communication lines.

A four-channel LIRA instrument monitors, by infrared, the carbon dioxide content in the exhaust ducts.

The airlock also has a spring-loaded hatch, ladder, complete sequential operating instructions on the wall for oxygen purge, depressurization and repressurization and a redundant dump valve on the inside. The valving system in the airlock is also color-coded -- "A" Yellow, for oxygen flush; "B" Red, for vacuum or vent; and "C" a fixed orifice repressurization valve that maintains a steady 4-minute descent time from 28,000 feet to sea level.

The occupational medicine aspects of altitude work require preoxygenation equipment consisting of demand-type oxygen regulators and walk-around oxygen bottles. A 60-minute denitrogenation period is required to prevent the occurrence of the bends while performing work at altitude. Standard Air Force oxygen mask with built-in microphone and the standard Air Force helmet with headset are used. When the technician enters the airlock he uses a walk-around bottle. He wears flame retardant coveralls and rubber boots. Inside the airlock, he reconnects to the permanent oxygen supply, purges the lock at ambient pressure with the doors closed until the oxygen concentration reaches 98%, removes the oxygen mask and starts depressurization. Upon reaching the altitude at which the dome operates, he opens the hatch and enters the dome via ladder and proceeds to clean and wash cages, feed animals or secure biological samples.

The outside observer uses standard Air Force communication gear with lip microphone, and must maintain complete visual and audio contact with the technician inside the airlock or the dome. Both the hard line and the wireless (walkie-talkie) communication systems are redundant and battery operated.

In summary, the toxicological experimentation supporting the development of space systems requires highly sophisticated equipment capable of simulating the atmospheric environment of space cabins.

Continuous, long term exposure alone opens up new concepts in experimental design; and, when combined with low pressure, oxygen-rich environments, the evaluations of combined stress, mitigation or potentiation of toxic effects by exotic environments become new and exciting challenges for the toxicologist. Solution of these basic problems more than ever requires a well organized team approach in order to fully evaluate the true physiological, pharmacological, biochemical, behavioral and aerospace medical aspects of space cabin environments.

TABLE I

PHYSICAL CONCEPTS

<u>CONTROLLED VARIABLES</u>	<u>UNCONTROLLED (BIOLOGICAL) VARIABLES</u>
Temperature	Species Sensitivity
Humidity	Indiv. Biol. Variation
Air-Flow	Pulm. Ventilation Rate (Total Dose!)
Pressure	Pulmonary Diseases
Atmosphere	Parasites
Composition	
Contaminant	
Concentration	

TABLE II

POPULAR CHAMBER DESIGNS

Bell Jars, Cylinders
 MacFarland (1m³ + Airlock)
 Public Health
 Rochester
 Altitude

CRITERIA

Practical Design
 Air-Tight Space
 Known Volume
 Ease Of Cleaning
 Corrosion Resistance
 Visibility
 Uninterrupted Exposure
 Altitude Capability

\$1,000
 IN
C
R
E
A
S
I
N
G
 COST
 \$100,000

TABLE III

CHAMBER EQUILIBRATION

$$t = K \frac{a}{b}$$

t = time K_{99%} = 4.6
 a = chamber volume
 b = air flow rate K_{95%} = 3.0

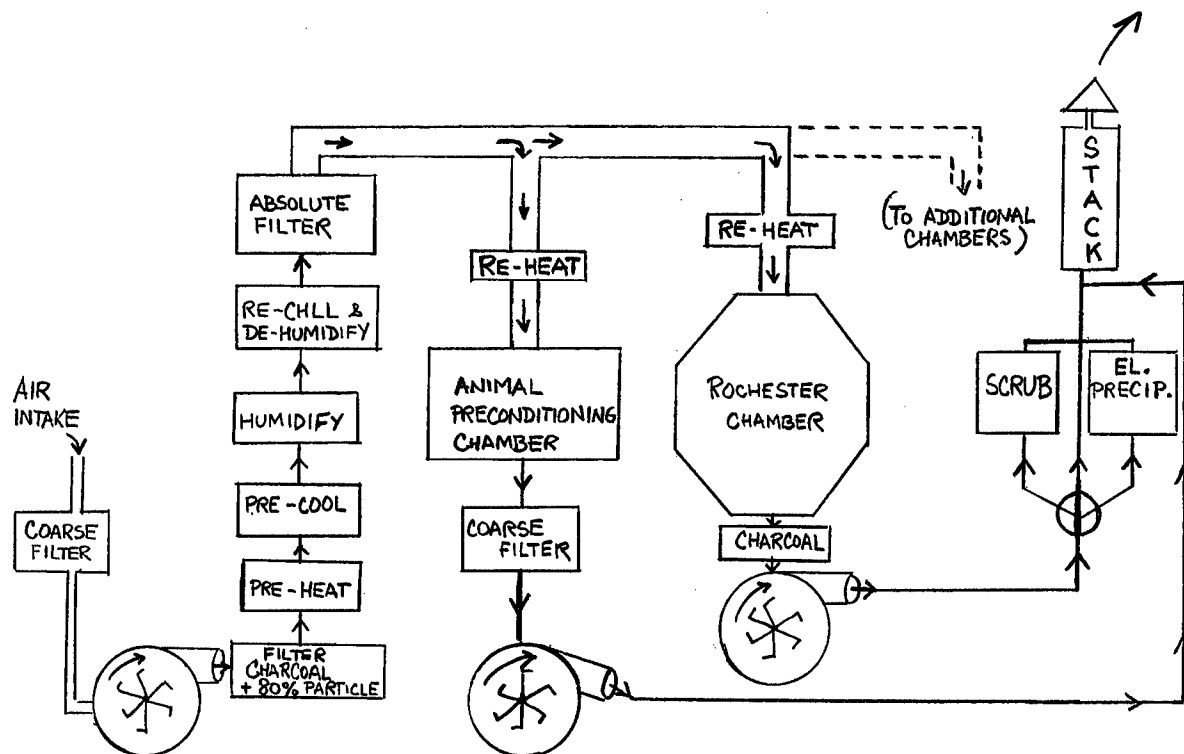


FIGURE 1. TYPICAL INHALATION EXPOSURE UNIT

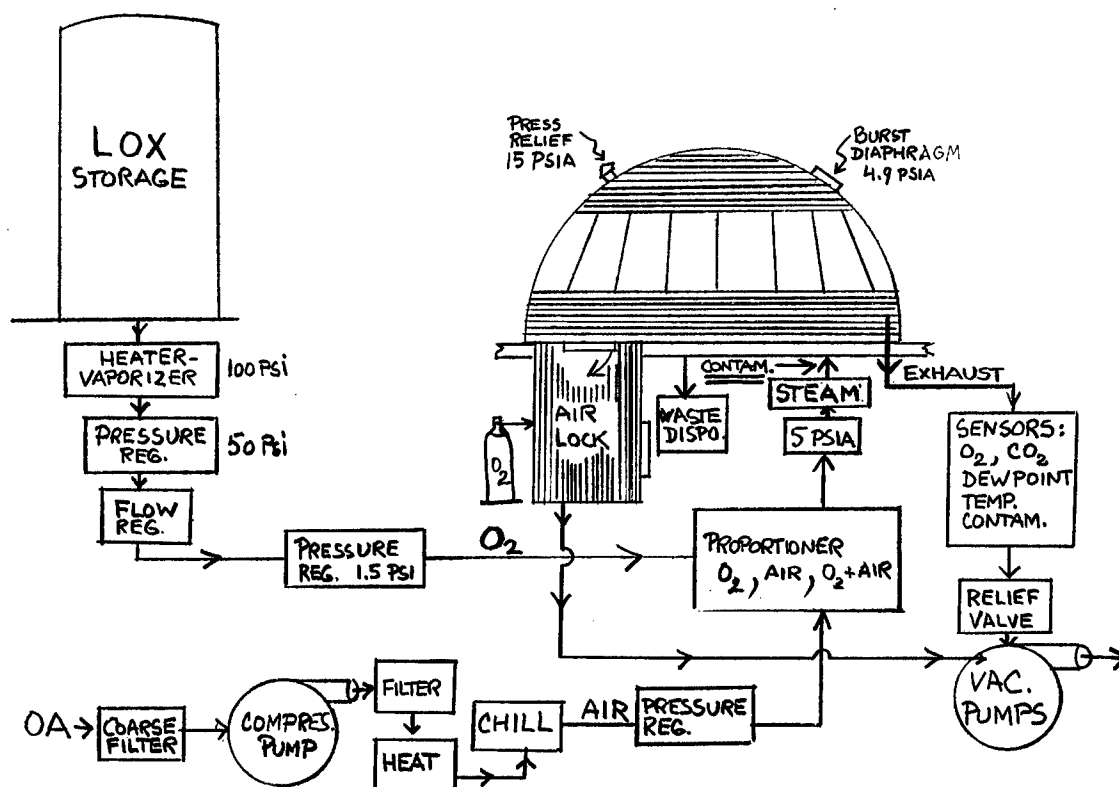


FIGURE 2. ALTITUDE EXPOSURE UNIT

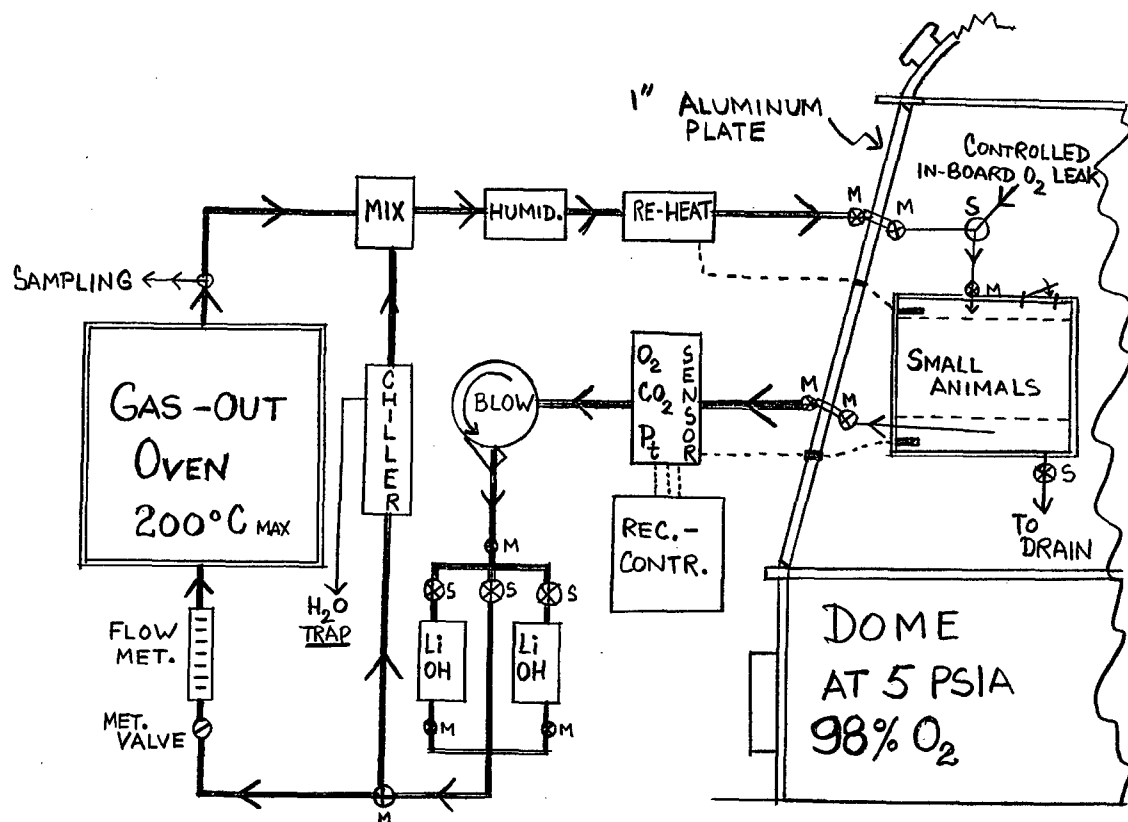


FIGURE 3. SCREENING OF CABIN MATERIALS
SIMPLIFIED FLOW DIAGRAM

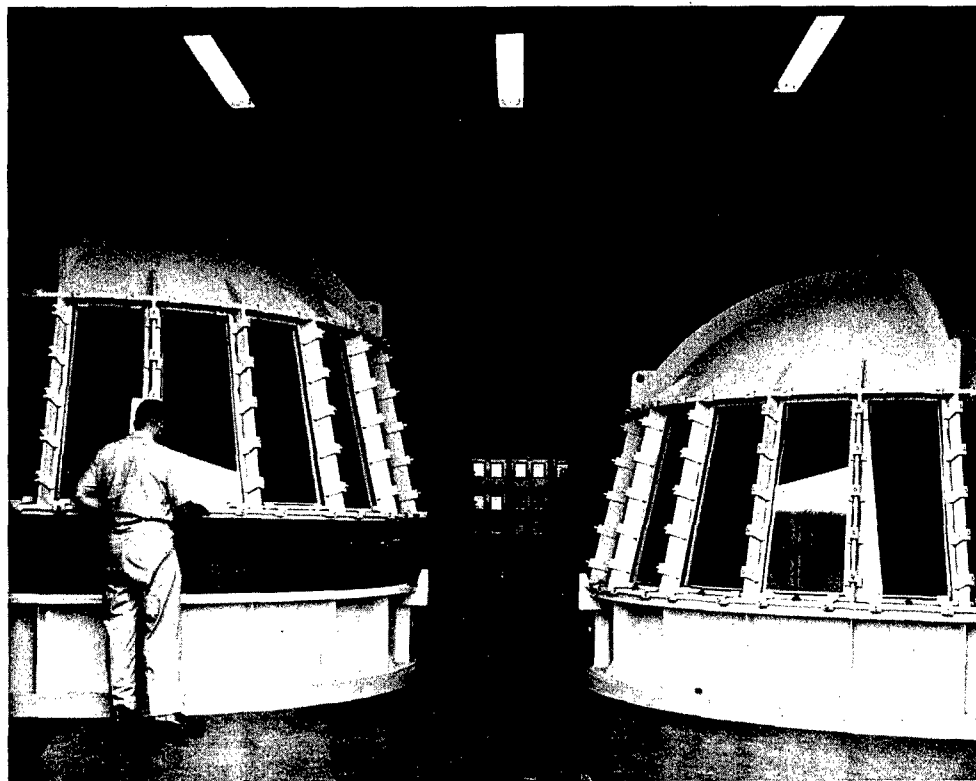


FIGURE 4. THOMAS DOMES

CONTAMINANT GENERATION METHODS AND TECHNIQUES

By

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The title of this paper is more comprehensive than the subject material which shall be covered. It is, however, broad enough to cover the three primary subjects to be discussed which are as follows:

- a. Generation techniques for single compounds or materials.
- b. Generation of gas-off products of mixtures of materials at reduced pressure in the presence of a 100% oxygen environment.
- c. A theoretical approach for establishing nominal chamber concentrations in the Thomas Domes described in the preceding paper ⁽¹⁾ at reduced pressure conditions.

Most of the methods which are described in this paper are not necessarily new methods. Indeed, many of these methods ^(2, 3) have been used in inhalation toxicology research for the past 25 years or more. Although the basic techniques may be old, the equipment used to implement these techniques in the Toxic Hazards Research Unit (THRU) laboratory are the best currently available.

For purposes of this discussion, the various types of materials, which are of interest in toxicology, may be subdivided by their physical properties; namely, gases, liquids and dusts. It should be pointed out that no classification scheme of materials to be tested will automatically identify the contaminant generation methodology required. Individual materials may, indeed, require new or modified techniques because of some unique physical or chemical property. Therefore, the contaminant generation techniques to be described in this discussion are for general case. Some of the more common problems that may require modification of contaminant generation methodology will be discussed.

GASES

Since many of the gaseous contaminants which are being or will be tested in the THRU laboratory are highly corrosive in nature, the entire contaminant generation system used is made of corrosion resistant stainless steel. This equipment is, therefore, useable for almost any gas and only in special circumstances would other materials such as monel metal be required.

Specific elements of generation of gases for introduction into an experimental chamber are pressure regulation, metering and flow control. In general, all of the currently planned research for the THRU laboratory entails pressures at or below atmospheric. Since gases stored in pressure cylinders require little impetus to initiate flow, the pressure regulation phase is the primary step in control of the desired quantity of material to be delivered to the animal exposure chambers. The pressure regulation is also necessary to assist in the metering of the desired gas flows.

The metering of gases in the THRU laboratory is conducted by one of two methods. Where possible, glass flowmeters of the rotameter type are used. When materials that are corrosive, to either the glass bore or the indicator floats of a rotameter, are in use, stainless steel laminar flow elements are the metering device of choice. The laminar flow elements, like the rotameter, must be calibrated for individual gases and require a readout device such as a differential pressure meter. In the THRU laboratory, the differential pressure measured across the laminar flow element is sensed by a differential pressure transmitter and recorded on a strip chart recorder. This enables continuous records of contaminant flow to the chambers to be obtained and preserved.

While most gases present no difficulties in pressure regulation and flow control, there are a number of special cases which have been shown by experience to cause special problems. These gases are those which have boiling points at or slightly below ambient temperatures. When the gas is released from the storage cylinder through metering valves, adiabatic expansion occurs with resultant cooling of the gas. This cooling produces liquified gas in the contaminant generation system which interferes with accurate metering and will result in fluctuations of chamber concentration. Two gases which meet this criterion are nitrogen dioxide and monobromotrifluoromethane.

The problem of liquification in the contaminant generation lines can be avoided by applying heat to the lines immediately following restriction points in the flow control devices. This heat can be applied by using simple heating tapes operated through a variable voltage transformer as shown in Figure 1.

In establishing a chamber concentration for an animal exposure chamber, the chamber air flow must be precisely known. The amount of gas or other material to be delivered and mixed with the chamber air stream can be calculated by the simple mathematical formula:

$$\text{ppm} = \frac{\text{mg/l} \times 24,450}{\text{mol. wt.}}$$

or, in the case of gases, by the more direct application of milliliters of the test gas per 1000 liters of chamber air flow. What has just been described is the establishment of a nominal chamber concentration. The establishment of a precise chamber concentration is dependent upon analysis of chamber air samples and the appropriate adjustment of either contaminant flow or chamber air flow, which are the two variables available for this purpose.

When extremely low chamber concentrations of gases are desired, it is frequently desirable to prepare more dilute gas mixtures than are commercially available or that may be available but are unstable for prolonged storage.

An additional reason for preparing dilute gas mixtures in our laboratory is that many of the gases to be studied are extremely reactive oxidizing materials representing a potential safety hazard. In order to obtain dilute samples of gases for introduction into animal chamber environments, a gas dilution facility was designed and developed at the THRU. This gas dilution facility is capable of producing gas mixtures of any required dilution, although normally 1% and 10% mixtures are prepared.

LIQUIDS

When considering the generation of liquid contaminants, they may be divided into two groups. These groups will contain either the volatile or the non-volatile liquid materials. The volatile materials are usually organic liquids of relatively high vapor pressure and low viscosity. The use of syringe type metering pumps, Figure 2, for the generation of volatile liquids is the method of choice in the THRU laboratory. This pump is a relatively smooth operating, gear driven feed device which results in reasonably uniform output for prolonged periods of time and permits the use of a large reservoir of the liquid under study. The instrument operates by causing one syringe to meter out its charge of the contaminant liquid while the second syringe is in the refilling cycle. When it is desirable to meter out extremely small quantities of liquid per unit time, motor driven single syringe pumps are available of similar design. Either of these pumps are used in conjunction with a vaporizing and air mixing chamber in which heat may be applied, if required, to insure complete volatilization. This vapor laden air stream may then be introduced into the chamber air supply in a manner to insure mixing and uniform chamber concentrations. The metering rate of these pumps is controlled by the motor speed and gear ratios selected for use and is capable of covering a wide range of contaminant flow rates.

The non-volatile or less volatile liquid agents may be introduced into an animal exposure chamber by the use of atomizers or fog generating devices. These are useful when materials such as oils are the agent to be tested. The generation of these materials, however, usually results in formation of liquid aerosols rather than vapor phase exposures to the experimental animals.

DUSTS

The most difficult materials for use in toxicity testing are the dusts. Although many methods of producing dusty environments in animal chambers are available, no single one is universally applicable. The insoluble dust materials may be introduced into an experimental chamber by use of the Wright Dust Feeder ⁽⁴⁾, shown in Figure 3. This instrument drives the dust filled container "A" down over the scraper blade "O" slicing off a small amount of dust into the air stream entering at point "H" and exhausting the airborne dust

particles at point "Q" for delivery to the chamber air supply. The most common difficulties encountered with this dust feed mechanism are plugging of the outlet jets if moisture or oil are found in the compressed air supply or if very dry air is not used with slightly hygroscopic dusts. Dusts which are very hygroscopic cannot be used satisfactorily with this equipment. The packing of the dust in the reservoir must be done carefully to make sure that it is packed tightly without air pockets. Improper packing will result in large fluctuations of the dust concentrations produced.

The dust feed mechanism known as the Far-Air Model Dust Feeder described by Baurmash et al ⁽⁵⁾ consists of a tray with four parallel grooves running the length of the tray. The tray is loaded with a weighed portion of dust spread evenly in the grooves between two marks on the tray. The chamber dust concentration is controlled by the number of grooves used and the amount of dust placed in the grooves. The tray is placed on the feeder device and advanced forward toward rotating brushes which sweep the dust into stationary aspirating tubes. The aspirating tubes are operated by an air supply regulated at 50 psig and are kept clean by a regulated rapping from pins located on the shaft turning the brushes.

Other methods of dust generation have included dust-sieve shakers ⁽⁶⁾, dust packed in tubes pushed by a rod into a compressed air stream ⁽⁷⁾ and conveyor belts from dust hoppers moving under an air jet ⁽⁸⁾.

Techniques for contaminant dust generation exposing only the head of the experimental animal rather than whole body exposures have been described by several authors ^(9, 10, 11). The advantage of this type of animal exposure is evident, in that the preening habits of many animal species are circumvented and large quantities of the dust are not ingested. Such ingestion could result in unwanted or uncontrolled systemic exposure to the dust under investigation.

Particulate aerosols can also be generated from suspensions or salt solutions by aspiration or entrainment devices such as those described by Dautrebande ⁽¹²⁾ and by standard DeVilblis nebulizers.

Generation of gas-off mixtures

A system has been designed for use in the THRU laboratory to simulate confined cabin exposures from mixtures of gas-off products resulting from reduced pressure and increased temperatures in a 100% oxygen environment. This equipment was designed to conduct experiments of the acute screening type. The schematic diagram, shown in Figure 4, illustrates the basic operating principle of the system. Mixtures of ten to 400 materials are to be placed in a constant temperature oven operated at 155°F and 5 psia (258 mmHg). Pure oxygen is to be passed over or through the test mixture which is then cooled to 60°F and excess moisture removed. Upon rewarming the contaminant laden air stream to 72°F, a 50% RH is achieved and the air will be passed into the animal exposure chambers. These small chambers are to be operated at

5 psia also and will be located within the Thomas Domes described in the preceding paper. The domes also will be operated using 100% oxygen at reduced pressure thus effecting a double envelope surrounding the animals under exposure. The air exhausted from the chambers will be passed through a lithium hydroxide scrubber system, to remove carbon dioxide generated by animal metabolism, and recycled continuously through the contaminant generation system and animal chambers. This recycling operation will produce the maximum possible exposure concentration for the evaluation of the acute toxic hazard resulting from inhalation exposure at reduced pressure to the mixture of test materials. The exposures will be operated continuously for a period of one week.

It is recognized that some of the gas-off products generated within the system may be lost during the air cooling stage and its passage through the lithium hydroxide scrubber system. This, however, is consistent with practical approaches to air cooling and CO₂ removal within space cabins and to the extent possible both test agents and scrubbing systems will be scaled proportionally to actual space cabin situations.

Establishing Nominal Dome Concentrations

In establishing nominal concentrations for exposure of animals in the reduced pressure chambers, it was quickly realized that the usual concept of parts per million would result in an effective concentration 1/3 the desired comparable ambient pressure exposure. That is to say, when the chamber was depressurized to 5 psia (1/3 atmosphere), a resultant expansion of the gases present occurred and only 1/3 the number of molecules of individual gases were present. Since parts per million implies parts of the test agent per one million parts of air, it was felt that the concept and nomenclature used in the reduced pressure experiments should be shifted to milligrams per cubic meter and to consider cubic meters of chamber volume rather than air. This concept is also more compatible with concentrations of contaminant as measured in a space capsule. A space capsule is essentially similar to a static chamber. An attempt will be made to simulate the same type of operation as in a space capsule by recycling the contaminant laden air in these experiments. Establishing concentration in terms of milligrams per cubic meter of chamber volume rather than parts per million would then result in an equivalent number of molecules of the contaminant material being presented to the respiratory epithelium of the experimental animals in both ambient and reduced pressure experiments. This concept is based on the assumption that tidal volume and respiratory rate are essentially equivalent under both experimental parameters.

In preliminary range finding experiments with these compounds, this approach seemed to be reasonably accurate and all experiments were subsequently conducted on this basis.

This modification in concept of producing chamber concentrations with respect to absolute chamber volume rather than percentage atmospheric composition

may be questioned in view of the possibility of altering pulmonary gaseous diffusion rates as well as pulmonary excretion rates. This possibility has been suggested since the total number of gaseous molecules present in the lung have been decreased by 2/3 when considering the reduced pressure experiments. Consequently, the ratio of contaminant molecules to other gases, in this case oxygen, has been increased.

Since there may be a possibility of altering the diffusion and excretion parameters, future experiments are being considered to investigate the extent of modification. It is possible that the study of pulmonary diffusion using various concentrations of carbon monoxide at both atmospheric and reduced pressure may help to resolve this question. It is also possible that the time required for equilibrium of blood carbon monoxide to be achieved at various concentrations of this material may be altered.

Summary

In summary, the contaminant generation methods used in the THRU laboratory have been described. Also, some of the difficulties encountered which required modifications of standard equipment for successful contaminant generation to produce uniform animal exposures in experimental chambers have been pointed out. The concept followed in the production of chamber concentrations at reduced pressure has been described in detail. The results of animal inhalation exposures conducted in this manner will be described in another paper to be presented at this Conference by Mr. McNerney. Additionally, a system which was designed to provide animal exposures to gas-off products of mixtures of contaminant materials at reduced pressure has been discussed.

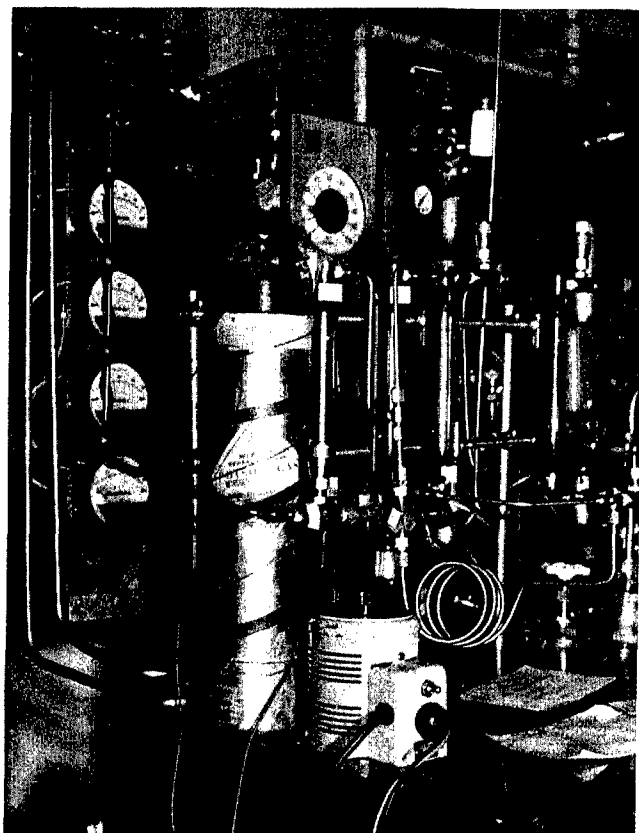


FIGURE 1. CONTAMINANT GENERATION OF NO_2 USING REGULATED TEMPERATURE HEATING TAPE

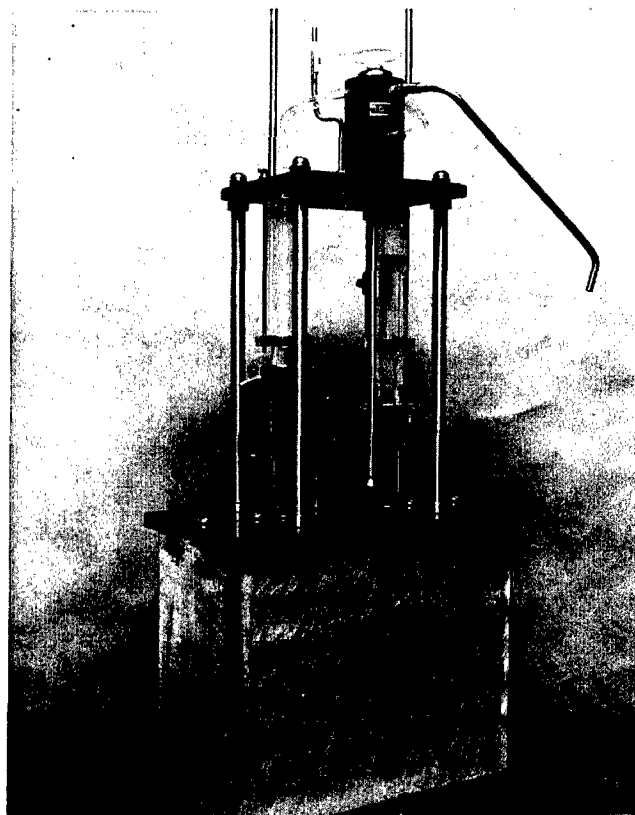


FIGURE 2. SYRINGE TYPE METERING PUMP

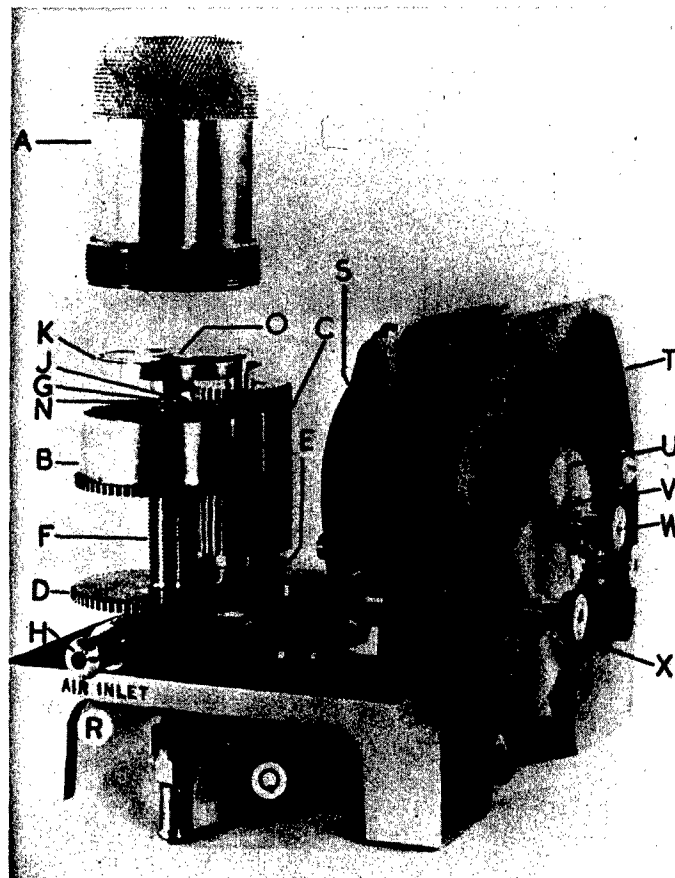


FIGURE 3. WRIGHT DUST FEEDER

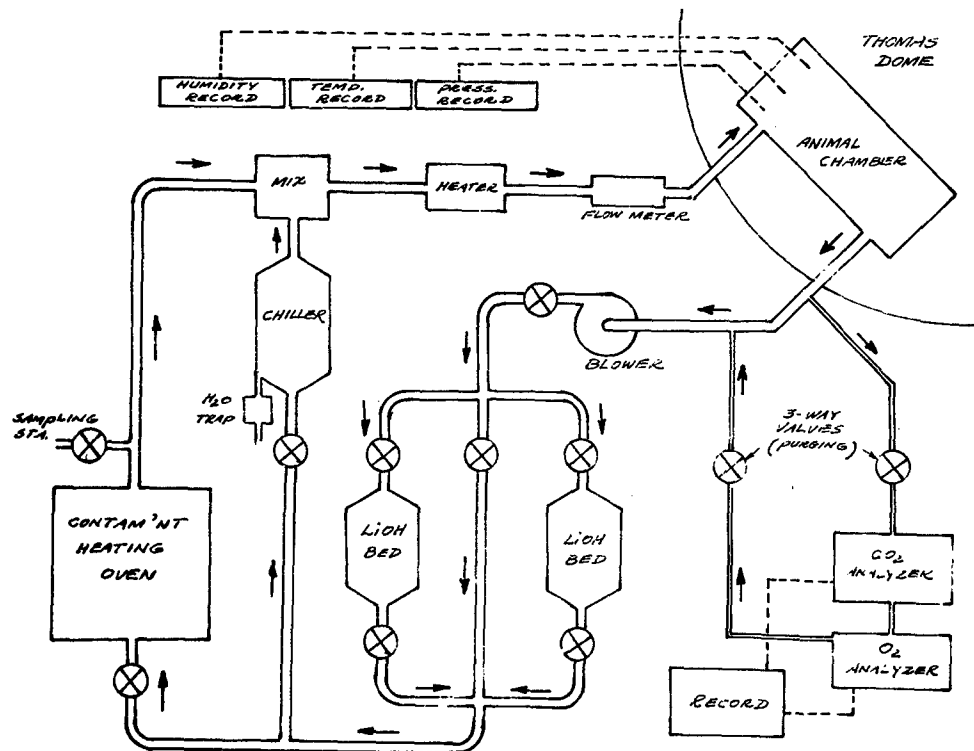


FIGURE 4. SYSTEM FOR GENERATION OF GAS-OFF PRODUCTS OF SPACE CABIN MATERIALS AT 5 PSIA - 100% OXYGEN

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ANALYTICAL CONTROL OF CONTAMINANT CONCENTRATION IN EXPOSURE CHAMBERS

By

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This presentation will outline the methods we have used at the Toxic Hazards Research Laboratory in the control of low concentrations of contaminants introduced into exposure chambers of various sizes. If the system could be depended upon to yield the calculated contaminant concentration in the chambers, there would be no need for control analysis. However, losses take place in delivery of the contaminant as shown by the fact that twice the calculated concentration of ozone, one and one-half times of nitrogen dioxide and 1.2 times of carbon tetrachloride must be introduced into our chambers to achieve theoretical concentrations. In addition, changes in the chamber atmosphere flow and in the contaminant generation equipment itself may lead to fluctuations in the contaminant concentration.

There is in the laboratory a good variety of instrumentation, including UV and IR spectrophotometry, gas chromatography and mass spectrometry, which might find applicability in control analysis of contaminant concentration. It was necessary to choose techniques for control which might be easily taught to chamber technicians with little or no background in chemistry. Spectrophotometry and gas chromatography satisfied this requirement and gave precise results in the hands of technicians.

The method of sampling is of first importance in the analysis of small concentrations of contaminants. After a short period of experimentation with different techniques, we selected pumping through a glass sampling tube or tonometer, and isolation of the sample after sufficient pumping for equilibration. The tonometer shown in Figure 1 has a sidearm which can be capped for sub-sampling by syringes or which may be used for the addition of reagent solution. When the sampled gas is at low pressure and must be brought to atmospheric before analysis, pieces of teflon are introduced into the tonometer to mix the sample with the added gas. This method of sampling has proved superior to fritted bubblers in our case for the following reasons:

1. The contaminant concentrations have been sufficiently high,
 (O₃, 1-10 ppm)
 (NO₂, 3-45 ppm)
 (CCl₄, 2-100 ppm)
 (CH₂ClBr, 500-1000 ppm),
 to make large volumes of sample unnecessary.
2. The volume of the tonometer can be measured exactly, whereas the measured flow rate of a sample through a bubbler is subject to uncertainty because of pump fluctuations. This error is amplified when pumping from pressures below atmospheric.
3. If the bubbler is not extremely clean, it may catalyze chemical change in a very reactive contaminant. Table I compares the apparent concentrations of ozone obtained by iodide oxidation in midget impingers, and after collection in a tonometer.

Table I

<u>Technique</u>	<u>Sample Flow Rate</u>	<u>Volume Sampled</u>	<u>Apparent O₃ Concentration</u>
Midget Impinger	1000 ml. /min.	10 liters	0.5 ppm
" "	" "	"	0.8 ppm
" "	" "	"	1.1 ppm
" "	" "	5 liters	1.9 ppm
" "	200 ml. /min.	2 liters	0.1 ppm
Tonometer	maximum pump speed	320 ml.	18.9 ppm

It is apparent that the cause of low ozone results in the midget impinger samples is not incomplete absorption in the collecting solution, since the lowest flow rate sample gave the lowest result. Evidence points to some mechanism which reduces the ozone before contact with the iodide solution or reduces iodine produced by ozone oxidation. In all the above experiments, 10 ml. of neutral iodide solution was used as the reagent.

A question which chemical analysis was called upon to resolve was whether the contaminants in the large "Thomas Domes" were being distributed uniformly over the volumes of the chambers. Accordingly, samples were taken in each of the domes at points which would reflect non-uniformity of concentration. Table II lists the concentrations so obtained.

Table II

<u>Dome Sampling Point</u>	<u>Contaminant Conc., ppm</u>		
	<u>O₃</u>	<u>NO₂</u>	<u>CCl₄</u>
Ceiling East	3.4	15.7	12.3
Floor West	3.4	14.1	13.2
Ceiling South	3.6	15.5	14.3
Floor Center	3.8	15.0	14.0

Considering the fact that these samples were taken manually using a rubber vacuum bulb, and the fact that the samples remained in the tonometer for a much longer than usual time (the technician had to carry them out through the dome locks), the results show reasonably uniform dispersion of the contaminants throughout the domes. Having established this, sampling points were placed at the most convenient sites, which proved to be in the atmosphere exit lines.

In the analysis of nitrogen dioxide, we used the method of Saltzman⁽¹⁾ with no change in the procedure. However, in the method for ozone^(2,3), we altered the standardization technique. Saltzman recommends diluting iodine solutions to very low levels for use as standards. Uncertain as to the stability of iodine at these levels during transfer operations, we made up our standards using potassium iodate to oxidize iodide in situ. Iodate will not oxidize iodide in neutral solution, but Kolthoff⁽⁴⁾ found that monobasic potassium phosphate is sufficiently acid to allow the oxidation to take place. Our work demonstrated that this oxidation was essentially complete after ten minutes, during which time no air oxidation of a solution without iodate occurred. After ten minutes, dibasic potassium phosphate was added to re-establish the conditions of the neutral phosphate Saltzman reagent. Standard curves obtained in this way fell within 10% of the data presented by Saltzman^(2,3).

Analysis of carbon tetrachloride in the large 800 cubic foot chambers and chlorobromomethane in smaller sixteen cubic foot chambers illustrate the use of gas chromatography in the control of contaminant concentrations.

In the quantitative analysis of a single component added to air or oxygen as in this case, the ordinary considerations of gas chromatography do not apply as strenuously as usual. Optimum parameters of flow rate, temperature and column packing do not have to be selected as carefully as when more complex systems are dealt with. The reason lies in the fact that no attempt is made to separate a complex mixture into its individual components but only to separate the contaminant from air or oxygen in a short

time and in such a way that some dimension (height or area) of the recorded peak so obtained will give a measure of the contaminant concentration with satisfactory precision. Since it is known that variability in flow rate and temperature lead to variability in peak height, these parameters once selected were reproduced as closely as possible in each run. The chlorobromomethane column, 1/4" I.D. 6' in length, with 20% SE-30 silicone grease on Chromosorb W, was chosen as one with intermediate polarity, and the temperature, 125°C, and flow rate, 80 ml./min., to elute the contaminant peak quickly. The concentration was satisfactory for thermal conductivity detection. Daily standard runs were made and the 95% confidence limits of a single determination calculated. Peak heights were used as a measure of contaminant concentration. Figure 2 is the curve constructed by averaging the results of a number of standard determinations. Peak height is seen to be linear with concentration. The 95% confidence limits of a single determination were calculated from ten standard analyses and found to be $\pm 7\%$ relative at both 500 and 1000 ppm. The actual precision of the analysis is thought to be even better than this, since much of the imprecision in standard analyses of gases lies in making up the standards. The most precise methods of making up low concentration vapor standards utilize rather complex flow dilution mechanisms. Since we had little time available for the construction of such equipment, our procedure was to inject the correct amount of liquid into a volumetric flask through a syringe cap, allowing to volatilize, and mixing. The volumes of liquid chlorobromomethane involved were quite small - 1.33 and 2.66 microliters respectively for 500 to 1000 ppm made up in one liter volumetric flasks.

In all quantitative gas chromatography of gaseous samples, we have used syringe introduction rather than sample loops and gas sampling valves. We have found the convenience and versatility of the syringe technique makes it preferable to the other methods.

As noted, the concentrations of chlorobromomethane analyzed were suitable for thermal conductivity detection. However, the concentrations of carbon tetrachloride introduced into the large chambers were too low for satisfactory measurement by this technique. It was decided to use electron capture detection, but this presented the problem of too much sensitivity. In order to get measurable differences between different concentrations of carbon tetrachloride, it was necessary to dilute the 30-100 ppm samples ten to one and introduce 5 microliter portions into the gas chromatograph. Under these conditions, the detector was measuring $1-5 \times 10^{-9}$ ml. of carbon tetrachloride vapor. Injections of such small volumes lead to error and, as a result, the CCl_4 peak heights were not reproducible. Fortunately, oxygen provides a reproducible signal in the electron capture detector and atmospheric oxygen could be used as an internal standard. Thus, the ratio of the CCl_4 to O_2 peaks was used as a measure of CCl_4 concentration, cancelling out any variations in sample volume. Data from a number of standards were analyzed statistically to give 95% confidence

limits of $\pm 18\%$ relative at the 80 ppm level. Although the true precision was probably better than this due to the errors in making up standards, a more precise method of analysis was sought.

Since most of the imprecision in the analysis could be traced to errors in dilution or in sample introduction, a method was sought which would permit the injection of reasonable volumes of undiluted sample. To this end a linear sample splitter was purchased, installed and experiments conducted on standards to determine the optimum combination of sample size and split ratio.

Figure 3 shows the experimental points of four standard runs using from 60-140 ppm CCl_4 using our final technique. The 95% confidence limits on the basis of these runs is $\pm 10\%$ relative, at 100 ppm, a good increase in precision over the previous method. Our splitter is started or stopped by inserting or removing a No. 20 syringe needle from the split septum. We allow the split to operate only as long as is necessary to achieve the split. Then we remove the needle, and all the carrier gas is directed through the gas chromatographic column. This preserves carrier gas and is necessary to elute the sample, since column flow during splitting is practically zero. The conditions of analysis were:

Column: 20% DC-200 silicone oil on Chromosorb W, 1/8"
x 6' SS tubing

Flow Rate: 80 ml./min.

Column Temp: 65°C

Detector: Tritium Electron Capture

Gas solid chromatography has permitted our laboratory to keep close control over leaks in the "Thomas Domes" when operating at 5 psi, 100% oxygen. We separate nitrogen and oxygen on a 5A molecular sieve column. Rather than oxygen, we measure the nitrogen concentration which reflects any air leaks into the system much more precisely than available oxygen monitors will. The nitrogen can be measured to within 0.25% at low concentrations and, since nitrogen is the only gas whose concentrations will increase to any significant degree in the dome during a leak, oxygen can be measured by difference with this precision of 0.25%. The recording polarographic oxygen sensing system installed in our system has a precision no better than $\pm 3-5\%$ relative and will not reflect small leaks.

All the analytical techniques described require relatively simple operations which a technician can carry out with ease and precision. With this system, we have been able to keep satisfactory control over contaminant concentrations in our exposure chambers.

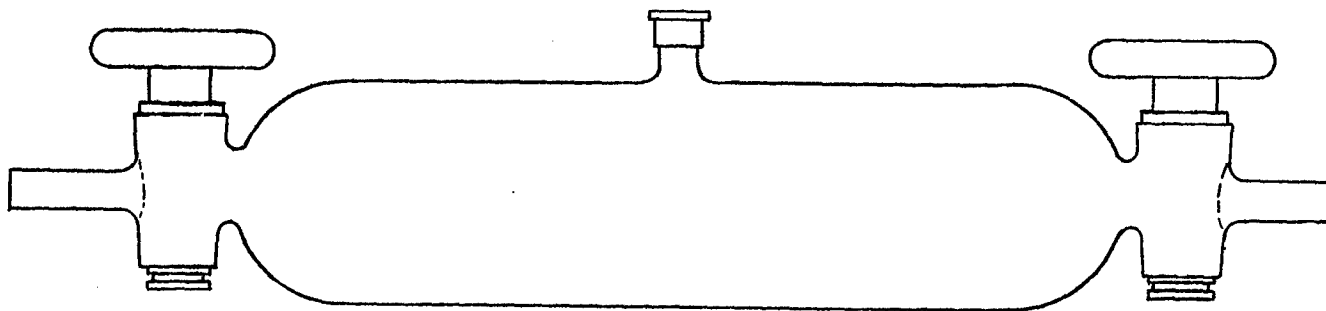


FIGURE 1. TONOMETER

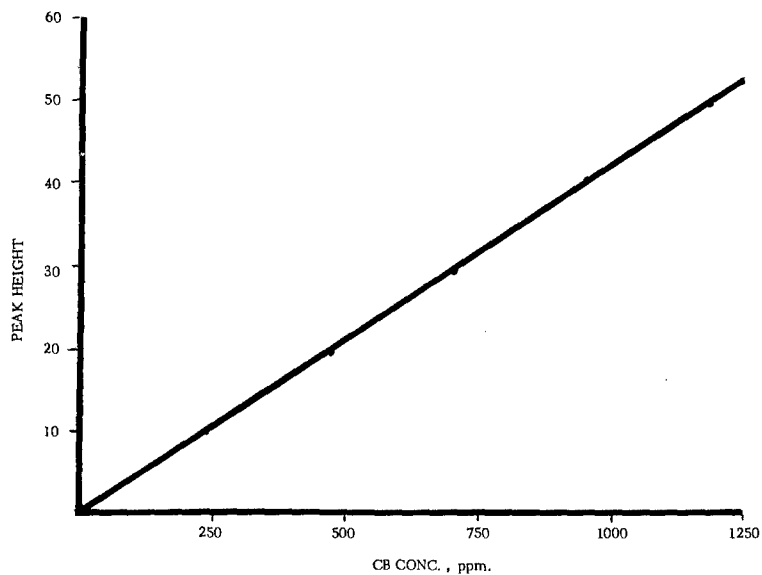


FIGURE 2. STANDARD CB CURVE

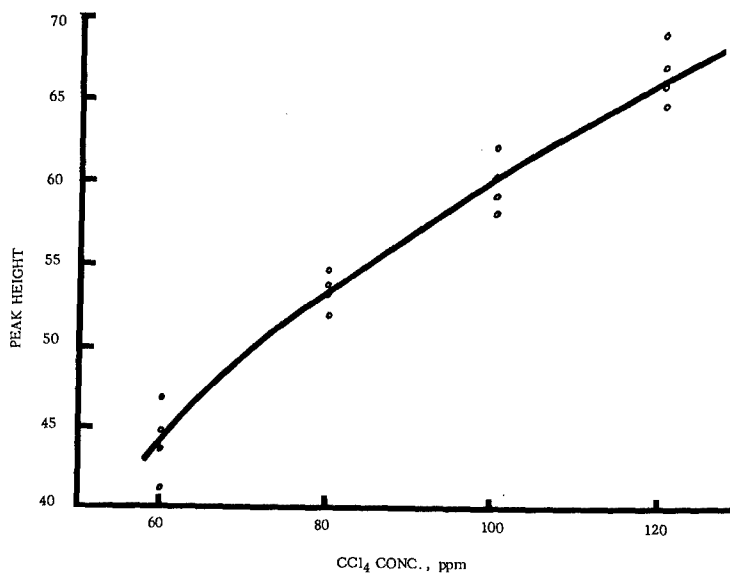


FIGURE 3. STANDARD CCl_4 CURVE

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THE NEED FOR CRITICAL EVALUATION OF CHOICE OF ANIMAL SPECIES FOR CONTINUOUS INHALATION EXPOSURE EXPERIMENTS

By

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INTRODUCTION

About 20 million animals are used annually, in the USA, for experimental studies initiated within diverse scientific disciplines. The choice of a mammalian species by experimentalists in the past has been decided by various reasons. It has been traditional, sometimes fortuitous, sometimes necessary and essential because of susceptibility to a particular infection, whilst economical factors have influenced the use of mice for acute toxicity assays. In other spheres, the use of one species has been on the basis of previous shrewd observations. Whatever reasons are found, it is doubtful if knowledge of spontaneous concurrent disease in any species, has influenced experimentalists. The theme of my talk is the reverse - namely whatever animals are used for chronic inhalation studies, there must be awareness of disorders which occur spontaneously and which could vitiate long term studies, involving pathology.

The concept that animals, used for experiment, should be bred under carefully controlled conditions, and be free from concurrent diseases, was brewed in earnest (first in Britain and later USA) during World War II, but there were lone wolves who thought on such lines long years before. Nowadays, it is considered an elementary accepted fact that only best quality animals should be used for experimental work, and the large commercial breeders in the USA have been prominent in their activities to achieve the impeccable goal. Rightly, or wrongly, the term SPF (Specific-Pathogen-Free) has been commonly applied to animals bred under defined conditions of isolation, with attention to balanced diets, to controlled environmental temperature and humidity and sound principles of genetics. For certain types of work, inbred strains are essential, and the use of germ-free animals comes into a special category but surely cannot be used for chronic inhalation studies. Part of the technique adopted for breeding healthy animals, has been to obtain breeding stock by cesarean section (better, post-hysterectomy delivered young), and fostering them on "germ-free" or "disease-free" mothers which need not be of the same species. The animals may be exposed to bacterial contamination later. Such a practice should, in theory, prevent the appearance of diseases due to transmissible infections from animal to animal, but we know certain conditions can be transmitted in utero, and crop up even in cesarean-delivered

stock (e.g. encephalitozoonosis in mice). The fact still must be faced that all highly practical procedures with rigid systems of control established in breeding operations, will not eradicate a host of other diseases such as the inherited, neoplastic or organic ones, or those due to hormonal imbalance, or the clinically silent lesions, which are found, incidentally, during thorough necropsy examinations in all species as old age creeps in. So, when any particular species is chosen for any long-term experiment, such as discussed at this meeting, it is a prime requisite for the pathologist to possess a sound knowledge of the diseases of the species used. The lesson will then be readily manifest of the need for caution when ascribing lesions found in an animal to whatever experimental procedure was followed during life. The literature contains many records of erroneous conclusions made exactly along this line, so that natural diseases have been described as experimental results.

The symposium is concerned with a discussion on all aspects of continuous inhalation exposures, and the more physical aspects are beyond my ken and interest. It is of vital importance, however, for the pathologist to be involved in such work. In the time allotted, attention has been confined to the respiratory system in the naive belief it is the most likely one to be affected in inhalation experiments. We are aware that inhalation of certain gases can produce hypoxia and a consequent severe degree of damage in the central nervous system. Similarly, I have restricted my choice of animal to the rat, monkey, dog and pig; time is insufficient to deal with the latter two, except to guide readers to the main literature. It then becomes obligatory to describe, and illustrate by lantern slides, certain diseases of the respiratory system of the rat and monkey to emphasize the absolute need to use animals bred under the conditions touched upon in my previous remarks. It is of little use to purchase "disease-free" animals and then not maintain them under the same rigidly controlled environment and conditions as practiced by the large commercial sources.

RATS

Factors, which have influenced the (now enormously increased) use of rats in experimental work, include their year-round high prolific rate of reproduction, their large litters, and their small size, all making for economy in maintenance and feeding and while using large numbers for any given experiment. Additional significant features (not often appreciated) concern the extraordinary toughness and resistance of the laboratory rat to infections. It probably suffers from far fewer natural diseases (of all kinds) than any other mammalian species - whether bacterial, viral, protozoal, helminthic, inherited, organic or nutritional - although the animal of choice for work in experimental nutrition; the rat is the common species used in a great variety of chronic toxicity experiments.

When we turn attention to conditions affecting the respiratory tract of rats, which could affect the results of chronic inhalation exposures, we are faced (fortunately) with a limited number of conditions. Acute bacterial

pulmonary infections in rats are very rare, but they can cause severe epidemics. The conclusion can be stated now that only SPF rats should be used. An incidence of moderate to high morbidity of the chronic murine pneumonia (to be described) in experimental rats could render evaluation of results - e.g. pathologic effects on the lungs - impossible.

CHRONIC MURINE PNEUMONIA

The name was given by Innes et al. (1956) to a disease in rats (and mice) as preferable to one in common use, enzootic bronchiectasis, which betokens only an end-stage of a disease which takes months to develop. In rats it is a disease with an insidious onset, slow protracted course and an inevitably fatal outcome, if the rats live on experiment long enough. Mortality at a young age is low but morbidity can be extremely high in a "conventional" breeding colony, and it is the outstanding hazard in chronic experimental work with rats and which may wreck experiments costing much labor, time and money. In some experimental irradiation work with observations on longevity, chronic pulmonary disease occurred in nearly 100% of 1-2 year old rats.

In earlier days, confusion was caused by the common isolation of Mycoplasma from infected lungs, and far too loose assumptions (by the inexperienced) were made on this microbial causation. Nelson showed that "bronchiectasis" was often a complex with another disease - "infectious catarrh" - with upper respiratory tract and middle-ear complications. He showed they were different, by first eliminating (in a rat colony) the latter without effecting incidence of the former. His work proved that the chronic disease was initiated in baby rats by a virus transmitted by the mother. Infection by direct contact from older rats to rats is possible if healthy animals are introduced into a colony where the disease is endemic. Thereafter, the disease process is a "slow infection". After establishment of chronic lesions with bronchiectatic cavitation (containing caseous material, cell and mucus detritus) there is secondary invasion by micro-organisms, such as Bacillus muris, Br. bronchiseptica, Pasteurella multocida muris, Streptobacillus moniliformis (Actinobacillus muris) and Mycoplasmatales. Nelson's original papers should be read for all work on etiology (all listed in Tuffery and Innes (1963)).

All who have had experience with this disease know that rats with such crippling destruction of lungs may not show tangible clinical ill effects, although clearly there must be some. In the late stages, the rats lose condition and weight, have a staring coat and there is snuffling and wheezing.

All lobes of the lungs (the single left one and four on the right) are equally prone. All right lobes may be totally consolidated and the left lobe left free. An affected lung is solid with a cobbled surface. The lesions lead to the formation of large nodular caseous or purulent masses in bronchiectatic

cavities, which are irregular cyst-like spaces, ultimately filled with caseous material, pus, detritus and mucus. Pleural adhesions or empyema are rare. The end process may be so severe as to present, on section, a whole lobe, or lobes, composed of multilocular cavities surrounded by collapsed lung parenchyma.

The genesis can be summarized. Lungs, from rats derived from colonies known to be free from this disease, histologically show little, or no, peribronchial lymphoid tissue. However, massive cuffs and sleeves of lymphoid tissue are visible in murine lungs after the disease starts and long before maturity and before there may be clear cut pneumonitis. Consequently, Innes et al. (1956) believed that the peribronchial lymphoid hyperplasia was the incipient stage of the chronic disease. Thereafter, in sequence there is interstitial pneumonitis, alveolar collapse, perivascular inflammation and bronchial fibrosis which all lead to increased rigidity of the bronchial tree and plugging of the latter leads to dilatation of distal parts (bronchiectasis). The occurrence of squamous metaplasia of the epithelium of the dilated bronchial channels is highly variable.

Innes et al. contended that rats are absolutely invaluable for experiment, but as long as the incidence of chronic pneumonia is high, they should not be used for experimental work in which pathologic effects on the lungs must be precisely determined. The presence of the various stages makes the task insuperable of differentiating between chronic lesions of a spontaneous disease and those produced by an agent and the problem becomes more onerous the longer the rats live. Unless the health status, and the condition of the lungs, of rats in colony is known, a diagnosis of chronic bronchitis being caused by any toxic (or infectious) agent is open to grave suspicion. Yet, there are papers on chronic toxicity tests in rats with this diagnosis. Some observers have admitted the presence of the same category of chronic pulmonary lesions in their control rats, but the fact has sometimes been conveniently ignored in their conclusions. This was emphasized by reports that chronic bronchitis ensued after exposure to diborane (B_2H_6), and McAdams reported work done to clarify the problem. Four groups of 20 rats were selected at ages of 5 and 9 weeks, 6 months and 1 year. In each group of 20, 10 were exposed to diborane (6 ppm), 4 hours a day, 5 days a week for 6 weeks, and 10 rats were kept as untreated controls. All animals were then killed. A critical evaluation was made of the pathologic status of the lungs; each possible alteration was graded as present or absent, or, when possible, quantitatively by figures from 1 to 4. An excerpt from McAdams' tabulated assessment is appended (Table I) to show how impossible it would be to deduce that chronic bronchitis could indeed be a toxic result. Reference should also be made to Ross and Jacobson on the toxicity of pentaborane, a study which involved examining 400 rats, and who came to the same conclusion. Indeed, qualitatively and quantitatively, pulmonary lesions were essentially worse in the untreated control rats than in those exposed to the diborane, so that the diborane (or pentaborane) might have been deduced to have had a therapeutic effect. However arbitrary the assessment of pathologic changes

(McAdams) might be, this is what might be constantly required in chronic toxicity work with rats not from a pulmonary-disease-free colony, if pathologic observations on the lungs are to have significance. This would require much time-consuming labor. The manifestly obvious solution is to work with rats from a colony in which chronic murine pneumonia has been eradicated.

INFECTIOUS CATARRH

Infectious catarrh is a chronic disease caused by pleuropneumonia-like organisms (PPLO:Mycoplasma) (See work of Nelson). It is widespread and found in most mouse and rat colonies not founded by techniques specifically designed to eliminate it. It leads to a general lowering of health, has a low mortality but high infection rate, which only may become evident in mice and rats examined at the end of a chronic experiment.

In mice, the most characteristic sign is a chattering or clicking noise, readily heard in a quiet animal room. As the disease progresses, particularly as pneumonia supervenes, the animal becomes less and less active and has a ruffled coat. Rhinitis, although usually present, never shows externally as secretions around the nostrils. Otitis media is usually detectable at necropsy but occasionally the labyrinth is involved and animals hold their heads in a lopsided position indicating interference with balance. Other affected mice and rats show circling or rolling, due to involvement of the middle ear and labyrinth, and there may be direct extension of the inflammatory process to meninges and brain. Rats never chatter, but they snuffle, and there are blood-stained nasal encrustations.

The lesions are confined to the nasal passages, middle ear and lungs. Rhinitis is indicated by profuse semi-fluid exudate in the nasal passages and which contains polymorphonuclear leucocytes and desquamated epithelium. It is also possible to demonstrate Nelson's "cocco-baccilliiform" bodies in or adjacent to cells in stained films. Otitis media shows exudate or pus in the middle ear cavity but requires microscopic demonstration, and infection may be unilateral or bilateral. Less frequently there is lung involvement - a slow, progressive bronchopneumonia with mucoid exudate plugging the bronchi. In such cases the chances are the animals may have had dual infection, Mycoplasma, plus the virus of chronic murine pneumonia (discussed previously), for in pure PPLO infections chronic pulmonary lesions may not occur (see in this connection Pankevicius, et al., 1957). Infection can spread from an abscess at the back of the throat and nasopharynx through the ethmoid bones into the cranium, middle and inner ear. A transverse section of the whole head through the middle ear is a good way of demonstrating the presence of such a disease process.

In old rats, routine histological examination of the lungs will reveal occasional granulomas with giant cells (unknown cause, or foreign bodies),

adenomatosis, pulmonary tumors, medial calcification of the main pulmonary trunks and curiosities.

SIMIAN PRIMATES

We start this section by the bald statement that under no circumstances can Macaca mulatta be used for chronic studies which necessitate critical evaluation of pathologic changes in the lungs, and for one very obvious reason - the universal infestation of the lungs of imported Macaca mulatta by the lung mite Pneumonyssus simicola. The solution to choice of simian primate, therefore, is to use Macaca bred only in primate colonies in this country, or alternatively use another genus in which mites and associated pulmonary disease do not occur. Any statement (which has been made in print) that the mites are not major pathogens is irresponsible.

Acute pulmonary disease (pneumonia) in apes and monkeys is a major cause of illness and death in imported animals, and can be caused by a variety of pathogenic bacteria, e.g. Hemophilus influenzae, H. bronchisepticus, Pneumococci, Pasteurella septicus, Past. pseudotuberculosis. Tuberculosis is a problem of great magnitude in laboratories using large numbers of imported monkeys. A variety of nematodes and trematodes can infect the lungs of apes and monkeys; hydatid disease has been recorded (for the simian species recorded as being infected, see Ruch for references). Several viruses have been recovered from simian lungs but their role is not well understood. Atypical (viral) interstitial pneumonitis associated with giant-cell formation and intranuclear and cytoplasmic inclusion bodies is well known (rather like measles), mainly in young animals.

It is too clear to those with experience in the pathology of monkeys born in a colony in the USA (where they are isolated from the mother at birth), and also of imported simians, that the pathology is entirely different. Indeed, the establishment of large simian primate centers has created new problems of disease both in nature and incidence, which are only beginning to be understood. There is no literature on the latter topic.

As has been mentioned at the beginning, for the purpose of this conference, we are concerned with chronic conditions which might interfere with the course of long-term inhalation work. We can thus confine our attention to the problem of lung mites (pulmonary acariasis), for it certainly is the most important one. As a condition, it poses an extremely interesting problem in biology and one which could have repercussions in human tropical medicine as well. Since becoming interested in the simian pulmonary acariasis around 1953, a few thousand monkeys have been examined by myself (or staff). If sufficient care is taken to look, it is safe to say that no imported Macaca mulatta will be found without some degree of mite infestation and lesions. On the other hand, in my experience, no macaca born in our laboratory, and isolated from the mother at birth, has been found on histologic search to show such parasites and lesions.

PULMONARY ACARIASIS - MONKEYS

The parasite was identified more than 60 years ago, and belongs to the genus Pneumonyssidae. Several different species of mite have been classified and the one involved in monkeys is Pneumonyssus simicola. Until about 1954, most reports dealt with single cases or very small numbers. The following is an amplified, but summarized, version by Innes et al. (1954), in which all important references to past work on the parasite and associated lesions will be found, and to papers on other mites infesting the lungs of seals, sea lions and snakes.

CLINICAL FINDINGS

By casual inspection of living monkeys, clinical signs of pulmonary disease may not be detectable, or may be only those of moderate attacks of sneezing and coughing. It is impossible to decide clinically whether any given living monkey will show no, a few, or disseminated pulmonary lesions at necropsy. The assumption must be that all imported adult Macaca mulatta do so.

It seems inescapable that mites, by their wanderings in the lungs, must irritate the bronchial passages and interfere with normal health, and reduce pulmonary capacity. Routine roentgenograms of the chests of monkeys in a normal colony are of no diagnostic value.

Since the pulmonary lesions are parasitic in origin, a prominent participation of eosinophilic leucocytes in the histologic reaction was suggestive that this might be reflected in an eosinophilia of the peripheral blood. This is not always evident. Complete routine hematologic examinations have been made (for other reasons) on very many monkeys passing through our hands. Any marked degree of eosinophilia, particularly that which can indubitably be correlated with the presence of mite lesions is not constant. The average eosinophil count might be 3.7 per cent in a total average white cell count of 12,000 per cmm.; a few monkeys showed a figure of more than 4 per cent eosinophils, these being 18 and 17 per cent respectively. In other animals, the average eosinophil count was 0.73 per cent in an average total white cell count of 21,200 per cmm., the highest eosinophil count being 4 per cent. Eosinophilia is thus never a constant finding, as it should be if it were related to the presence of mites in the lungs; but, even if present, other internal parasites, e.g., the common helminthic infestations in the alimentary canal, might be responsible.

PATHOLOGY

Macroscopic Findings

The parasitic nidus with inflammation is discrete, and may seem to be almost always subpleural, but this is not so. If the lungs are fixed and then

sliced, foci may be found in any part of any lobe. They may be just within macroscopic visibility as pale yellow spots, midst normal pink, crepitant lung tissue. Most range from pinhead size up to lesions a few millimeters or more in diameter. Superficially, they simulate some hematogenous mycobacterial lesions, but they are not firm like tubercles. Infrequently they are surrounded by a congested zone. Under the dissection microscope, foci are jelly-like masses in the centers of which is a minute slit, or round irregularly enlarged opening, or appear as a small cyst. In the foci there is an irregular marbling effect produced by the presence of black pigment and which forms a ramifying network throughout adjacent normal lung - just like an anthracotic lung. Many lesions become confluent, but rarely the process spreads by contiguity to result in a lobar pneumonia. A few monkeys show extensive lobular areas of consolidation and very many have multiple "violin string" fibrous adhesions to all pleural regions. The regional (hilar and bifurcation) lymph nodes show pigmentation which is not anthracotic. We have counted many dozen to 100 lesions in all lobes many times. The causal mites migrate in a somewhat fortuitous fashion to any part of the lungs.

Microscopic Findings

The pulmonary foci have a classical structural pattern, which cannot be confused with any other focal, or disseminated, disease of man or lower animals. Most lesions have a connection with a bronchiole, and beyond a level in the tree at which cartilaginous plates disappear. In some lesions, they are embedded deep in lung parenchyma and not involving bronchioles - large, small or terminal - but this probably is a matter of the level at which the lesions have been cut, as we have found in serial sections. The developed process is one of restricted bronchiolitis and peribronchiolitis, which directly involves only adjacent alveoli to cause some lobular collapse. The mite causes inflammation leading to a thickening of the bronchiolar wall with variable destruction of the lining epithelium, a reactive hyperplasia with lymphocytic aggregations in the wall, and the small macroscopic slit or hole seen in the center of the advanced lesion is a constricted bronchiolar lumen. Only in few instances can the lesion be referred to as a walled-off subpleural cyst. The latter word may be a little misleading, for the so-called cyst is simply a dilated segment of the wall. When fully developed, the bronchiolar lesion has an enormously thickened wall, and the lumen is reduced to a crevice (partly by detritus and mucus), in the midst of which lie the parasites, but in single sections only a part of the head, legs or body may be seen. The parasite readily falls out in preparation of paraffin sections, leaving an irregular ectasia, or the latter may be a late intrinsic feature of the lesion, for the detritus and mucus may be coughed up. Great destruction of the lining epithelium occurs with the mucosa stripped down to the muscle layers. The thickening of the wall is largely due to inflammatory cellular exudate in which there are polymorphonuclear and eosinophilic leucocytes, lymphocytes and macrophages; the number of eosinophils varies from lesion to lesion but may predominate. Peribronchiolar lymphoid infiltrations may be pronounced, with

formation of prominent follicles. The distribution of the pre-existing muscle fibers of the bronchiolar wall may be irregular, but this perhaps is a matter of displacement of the fibers within the area of inflammation. Some true proliferation of plain muscle fibers has been observed.

Scattered throughout the lesion are macrophages crammed with golden brown to blackish pigment and refractile crystals (Charcot-Leyden) which form dense clumps in, near and around the main focus. The pigment and crystals can be scattered far and wide throughout lung tissue, distant from any parasitic focus, but is then found mostly in phagocytes within alveoli, perivascular and peribronchial lymphatics. Occasionally there are aggregations of pigment-bearing phagocytes lying free in otherwise normal air spaces, and some pigment may form extracellular masses. There is little or no tissue necrosis and no giant cell formation. Fibrosis is not a cardinal feature to the extent of forming dense collagenous bundles, but special stains reveal an anastomosing fibrillary recticulum ramifying through the bulk of the thickened inflamed bronchiolar wall.

In regions of focal lobular pneumonitis there is alveolar collapse, or the air spaces are consolidated and filled with exudate composed of all types of leucocytes, sometimes with eosinophils predominating, and pigment-bearing macrophages. This focal pneumonitis without any mite may be the lesion found incidentally in monkey lungs.

Lesions have been found which do not fit into the general pattern of the changes described. These were found in lungs in which there were relatively few macroscopic foci, hence such lesions may represent early stages of the process. The respiratory bronchioles and alveolar ducts, in very restricted segments of the bronchial tree, showed a striking degree of ectasia and far less inflammation. There is no metaplasia of the alveolar epithelium such as is seen in jaagsiekte, or in other chronic inflammations of the lungs of some domestic animals (including those caused by nematodes).

The pigment, characteristic of the lesions in simian lungs, deserves particular reference, for it is a diagnostic feature. Monkeys may acquire some anthracotic pigment, so all pigment present in any one monkey lung cannot be deduced to be of the Pneumonyssus variety. The latter has usually been considered to be the fecal excreta of the mite which has ingested blood from the host and some is hemosiderin. The nature of the crystals is unknown, but they are birefringent, and probably are Charcot-Leyden crystals.

Pulmonary lesions, and the mites, have been found in monkeys which have died within a few days of arrival, and in some killed on receipt within as little as 7 days after leaving India. It is certain that the animals acquire their infestation in their native country - India, Burma, or elsewhere in the Far East. Beyond that, there is little or no information regarding the

life history of the mite and the pathogenesis of the disease. How, where and when they acquire their original infestation can only be surmised. Nor do we know at what age they are first exposed and susceptible. We do not know how, or indeed whether, infection can be passed from monkey to monkey, but it is highly likely. Although in many lungs it might seem that all lesions by their size are at the same stage of development, it is difficult to believe there cannot be repeated reinfections. Monkeys supposedly could pick up these mites from grain or vegetation or other monkeys in their natural habitat, but the mites seem so susceptible to lack of moisture that it would be surprising if they were able to live apart from an animal host. We have not found Pneumonyssus on the skin of monkeys. It is doubtful that they are ectoparasites which accidentally find their way into the lungs. Some workers have suggested that the ova are swallowed, and that after hatching the larvae pass via the lymph-vascular system into the lungs. This is unlikely, for the size of the larvae would prevent them from getting very far in the vascular system without causing infarction. A more plausible explanation would be that the mites enter into the nasal cavities, and reach the lungs by direct wandering down the airways and mites have been observed in the air sinuses of monkeys by Oudemans. All stages of the parasite have been found in the pulmonary lesions of monkeys, viz., adult males and females, eggs and larvae. Consequently, the total life cycle could be passed within the lungs. Cases showing multiple disseminated lesions could thus represent not only new exogenous infections from coughing and by nose and mouth contact from other affected monkeys, but also repeated endogenous reinfections by fresh hatchings, larval maturations and new migrations.

Mite infestations of the lungs by Pneumonyssus spp. are stated to be found only in old-world monkeys. Because of the very high incidence, it cannot be ignored that other animals (laboratory and domestic) kept on the same premises might become infested. We isolated adult mites, introduced them into the trachea of a rabbit, and found a few acari still alive in the lungs after 12 days, although whether true lesions, as seen in monkeys, were produced was questionable.

This exemplifies one important point, namely, that the parasites can survive in the lungs of abnormal hosts for some time, and that cross-infestation between monkeys and other mammals (even man) may be possible. The finding of mites (not necessarily Pneumonyssus spp., but Tyroglyphus and Tarsonema) in human sputum suggested possible causes for certain tropical pulmonary disorders such as asthma, bronchitis, and Loeffler's syndrome (tropical pulmonary eosinophilia), the cause of the latter being still unknown.

DOGS AND PIGS

There would be many advantages in using dogs, preferably small ones such as beagles or corgi, for chronic inhalation work. Good clinical observations can be made, and there is a vast amount of knowledge available in the

literature on the pathology of diseases which affect the respiratory system. The same applies to the domesticated swine. Colonies of both species have been raised as SPF stock. For reference to pathology, see Smith and Jones (1961), and Jubb and Kennedy (1963), while Dunne (1964) has edited a text-book dealing exclusively with diseases of pigs.

TABLE I

(Excerpt from McAdams, 1955)

Lesions	Control rats					Experimental rats				
	1	2	3	4	5	1	2	3	4	5
Lymphoid infiltration										
bronchial tree										
Mucosal	4	3	4	2	3	3	3	3	1	4
Disruption of basement membrane	4	3	4	2	4	3	3	3	1	4
Submucosal	4	3	4	3	4	3	3	3	2	4
Bronchial musculature	4	3	4	3	4	3	3	4	2	4
Follicle formation	+	+	+	+	+	+	+	+	+	+
Limited to large bronchi	4	3	4	3	3	3	3	3	2	4
Limited to small and medium bronchi	4	3	4	4	4	3	3	3	2	4
Limited to bronchioles	3	1	4	1	±	1	1	4	2	1
Extracartilaginous	+		+	+	±	0	+	0	0	+
Perivascular lymphoid tissue										
Large vessels	2	1	2	1	2	2	1	1	2	3
Small vessels	4+	2-3	2-3	2	4	3	2	3	3	2
Thickening of alveolar walls										
Inflammatory	+	+	+	+	+	+	+	+	+	+
Hyalin										
Patchy	+	+	+	+	+	+	+	+	+	+
Diffuse, local	+	+		+		+		+		
Diffuse, general										
Content of alveolar spaces			0							
Acute exudate						2	2	2	1	
Chronic exudate	2	1		1	1					
Macrophages						3	2	2	2	1
Patchy	+	+		+	+	+	+	+	+	+
Diffuse, local										
Diffuse, general										
Pleural thickening	0	0	0	0	0	0	0	0	0	0
Fibrosis	0	0	0	0	0	0	0	0	0	0
Focal										
Diffuse, local										
Diffuse, general										
Bronchial dilation	4	2	3	2	1	1-2	0	0	0	4
Bronchiolar dilation	2-3	1	4	0	0	0	0	3	0	0
Exudate in bronchial tree	+	0	+	+	0	+	+	+	0	+
Destruction of bronchial epithelium	+	0	+	±	0	0	0	0	0	3
Epithelial regeneration	+	0	+	2	0	1	3	1-2	0	0
Epithelial hypertrophy	3	0	3	2	2	1-3	1	3-4	0	3

— = not in section.

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CLINICAL LABORATORY METHODS

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In the selection of specific clinical laboratory methods to recommend for use in continuous inhalation exposure studies, consideration has been given to economy, ease of performance, speed of obtaining results, reproducibility or precision, sample size required, inherent laboratory error and general acceptance of the specific principles involved. I wish to stress that in presenting these methods, I am not implying that these are the biological parameters which should be monitored; only that, should you wish to determine any of these particular components, these are methods which I recommend. Some importance has been attached to specificity in certain instances although this desirable characteristic becomes increasingly more difficult to establish as is evidenced by the continuing stream of publications of new methods and new modifications of old methods.

Most of the methods which I have chosen to discuss involve colorimetric analysis and measurement by spectrophotometry, since adequate and reasonably priced instruments are universally available and are easy to operate with a minimum of maintenance. In connection with the use of colorimetric methods, there are several fundamental rules which must be strictly adhered to and which will bear frequent reiteration. The most important consideration must be given to colorimetric reactions and deviations from Beer's Law. These deviations are both frequent and significant. The law is valid only over a limited range of concentrations, even under the most ideal conditions, and conformance to Beer's Law must never be assumed but should be checked in each case under the identical conditions of the adopted procedure, for the concentration range to be met with in practice. A minimum of 3 points is required, one representing the highest concentration anticipated, and one giving zero absorbance for zero concentration. If a straight line relationship does not exist between intensity of color and concentration, a minimum of 8 points should be established and the curve so drawn used for reading unknown concentrations.

Absorbance readings on spectrophotometers vary significantly from instrument to instrument -- as much as 20% for the same make -- and some arbitrary standardization check must be made frequently. A suitable standard for this purpose is the potassium chromate standard for icterus index which is stable for many years.

Cuvets must be individually calibrated and given proper care, avoiding scratches and fingerprints. They should be oriented within the cuvet well at that position giving maximum transmittance of light and be properly marked.

In the reading of results, zero absorbance should be set with water and a reagent blank should be used. Ideally, one would adjust the procedure so that the intensity of color for the desired concentration range will permit reading of the reagent blank as well as unknowns against water. This allows for a daily check of reagents and some quality control. If it is necessary, as with a colored reagent blank, to use this as the zero absorbance point, one should still not neglect daily checks of the reagent blanks against water.

Dilution of the final color should never be done unless the validity of this procedure has been established by experimentation. Measurements should be confined to the range of 10-80% Transmittance (Absorbance 1.0-0.11) if possible.

The terms macro, micro and ultramicro are defined in terms of the amounts of sample required for analysis. Macro refers to volumes of 1 ml or more; micro to approximately 0.10 ml; and ultramicro to 0.010 ml or thereabouts. Most macro methods can be scaled down to micro proportions by starting at the end and working backwards, limited only by the minimum final volume needed and the spectrophotometric equipment at hand. Tests on an ultramicro scale require special micro apparatus and they are recommended for small animal work with the stipulation that results be directly comparable to reference macro methods.

Control of laboratory error must be constant. There are three types of standards commonly in use today - the pure standard, the internal standard and the pooled sample or commercial lyophilized serum. It must be remembered that the latter are "secondary standards" since the stated values for the various substances are arrived at by referee laboratories. Furthermore, the stated values may be valid only for the methods used by the referee laboratories. At any rate, the use of both pure standards and serum controls is recommended routinely for all clinical chemistry determinations. Quality control charts should be standard practice for any laboratory.

Most chemical analyses can be performed equally well on plasma or serum. If plasma is to be used, heparin is the anticoagulant of choice, at a concentration no greater than 0.20 mg/ml blood. One precautionary comment should be made regarding the use of heparinized plasma for inorganic phosphate determinations. It appears that most commercial heparin is contaminated with some phosphate and will, of course, give spuriously high results.

For enzymes, electrophoresis, and for glucose, preservation of samples by any means is not recommended. Separation of cells and serum should be made within 30 minutes of collection, and analysis should be carried out, or

at least started, within two hours. If this is not possible for all determinations, judicious choice must be made of those procedures that should be done first. The time elapsing between sample collection and analysis should be not only noted but also reported, so that comparisons between laboratories may be properly evaluated. Table I shows the experimentally proven stability of several serum components. Those with doubtful stability, either at refrigerated or frozen temperatures, are obviously the determinations which must be performed without delay.

Table II is a list of the more common biochemical serum components along with the general principles of the recommended methods. A detailed reference list of methods has been prepared as a handout and copies are available at the Chairman's table following this session.

I shall attempt briefly to explain the selection of specific method where a multitude of acceptable methods exists. No hematological parameters such as erythrocyte or leucocyte counts, hematocrit, differential enumeration, reticulocyte counts or calculation of various indices have been listed since these are standard procedures to be found in any good teaching or reference text. In passing, however, I should like to recommend strongly the routine use of a Coulter Counter, microhematocrit determinations, and the Wright-Giemsa stain for white cell differentiation.

The choice of the Ferro-Ham method for serum calcium is primarily based upon a need for reproducibility from analyst to analyst. This method has a precision of $\pm 2\%$ and results are quite comparable to the titration reference method of Clark and Colipp which, incidentally, has a precision of $\pm 3\%$. The Ferro-Ham method is relatively rapid and has the added advantage of usefulness for urine and feces as well as blood serum. This procedure is one which is particularly useful for work with young experimental animals because linearity covers a wider range than other colorimetric procedures. This is important when normal baseline values tend to exceed 5.5-6.0 meq/liter, as is the case with extremely young animals.

My natural antipathy for clinical chemistry methods involving titration techniques is somewhat overcome by the sharp, visible end-point, precise within very narrow limits, in the chloride method of Schales and Schales. It is good practice, however, to effect the prior precipitation of protein.

The method of Zak for cholesterol and cholesterol esters is recommended, again primarily because of its precision ($\pm 5\%$). The importance of reproducibility cannot be overemphasized.

Glucose oxidase is relatively specific for the oxidation of β -D- glucose to gluconic acid and allows measurement of all true glucose when the glucose oxidase preparation contains also the enzyme mutarotase, which must be present for conversion of any α -D- glucose to the beta isomeric form. The

use of a Somogyi filtrate, a zinc sulfate-barium hydroxide protein precipitating agent, also insures exclusion of interference from hemolysis, icterus, uric acid or other carbohydrates.

Although total serum lactic dehydrogenase levels by chemical means appear to provide reasonably narrow limits of normal range for humans, our experience with various animal species indicates that such a wide intra-individual variation occurs that results are extremely difficult to evaluate in terms of any toxicological response. On the other hand, the LDH isoenzyme patterns appear to be quite constant. Therefore, discontinuous gel electrophoresis is recommended as the method of choice. Details of this procedure can be obtained from personnel at the Toxic Hazards Branch when you visit our facility tomorrow afternoon.

Although the use of β -glycerol phosphate as substrate for phosphatase activity has long been the "reference" method, there has been much modification, some confusion, and little agreement among investigators. The measurement of inorganic phosphate released from this or other substrates is the basis for the majority of these methods. Since, in most toxicological studies there is seldom any real indication for inorganic phosphate determinations, a method eliminating this step would allow for more rapid analysis and also use of smaller sample volume. Therefore, the method of Huggins and Taladay* as modified by Klein, Read and Babson is the method of choice. This method involves the simple release and measurement of phenolphthalein from a phenolphthalein diphosphate substrate. Since the reagents for this test are commercially available, it is an extremely simple test to perform with maximum control of laboratory error.

Due to the very real problems of instability associated with the use of p-aminonaphtholsulfonic acid as the reducing reagent in the "standard" method of Fiske and Subbarow for determining inorganic phosphate, the substitution of a more stable agent, p-methylaminophenol sulfate (ELON), results in a more reproducible and predictable method. However, a word of caution is in order regarding the use of the trichloroacetic acid (TCA) deproteinizing reagent. As shown by Gomori* the ratio of acid to molybdate is extremely critical and therefore any deviation from the recommended dilution of serum with acid or any arbitrary change in TCA concentration is to be condemned.

Since every well-equipped clinical and research laboratory today should have an electrophoretic apparatus, there seems little reason to continue the practice of determining A/G ratios by salt precipitation methods. With the advent of cellulose acetate (Sepraphore) papers and reasonably priced horizontal

* Huggins and Taladay, J. Biol. Chem. 159: 399 (1945)
Gomori, J. Lab Clin. Med. 27: 955 (1942)

cells for separation, as well as integrated scanning devices for measurement of dye binding capacity, the entire separation of protein subfractions can be accomplished in 3 to 4 hours. For the added information gained by this method, this amount of time seems entirely justified. The total protein determination involves the classic Biuret reaction.

The measurement of colored end products from the transamination reactions has become an accepted and routine procedure in nearly all hospital clinical laboratories. While the colorimetric procedure is not an absolute measure of accuracy, as is claimed for measures of reaction rates, it has the advantage of reproducibility and ease of performance with a minimum of inter-laboratory variability. From the standpoint of the human clinician, it matters little what transaminase method is used or what results are obtained by any laboratory other than the one responsible for the work on his own patient. In the case of experimental toxicological research, however, valid comparability of data from laboratory to laboratory is a necessity for proper evaluation of the slight to moderate biochemical changes which may occur. The Reitman-Frankel method appears to lend itself best for this purpose, and reliable reagents are available commercially. In terms of time required to complete an analysis for transaminase, there is no doubt that the colorimetric procedure recommended is less exacting and less time consuming for a busy laboratory.

The use of urease and subsequent nesslerization of the released ammonia from urea nitrogen is probably still the method of greatest specificity. Since absolute specificity is the ideal approach to any clinical chemistry method, this classic method is recommended. Strict adherence to procedural detail will provide excellent and reproducible results.

Flame photometry is the most sensible and easiest method of analyzing for sodium and potassium. No specific methods have been recommended since these are dependent upon the particular instrument available, and all methods give comparable results. Since methods for determining calcium by flame photometry are still in a state of constant review and discussion among clinical investigators, the routine use of flame photometry for serum calcium is not recommended at this time.

If the bromsulfophthalein dye retention test is contemplated as a test of liver function, we recommend use of a 10 mg/kg dose and a 15-minute sampling time. In dogs, at least, (the species most likely to be used for this type of test) the extremely rapid excretion rate of BSP dictates that this increased dosage be used. The presence of this dye in the serum will, of course, interfere with practically all other chemical and hematological determinations listed, and experimental protocol must take this into consideration.

Table V illustrates an additional variable which may be standardized by mutual agreement among those involved in the laboratory aspects of continuous

inhalation exposure experiments. Reporting units usually follow the system which has been learned and passed on from one investigator to another within a single organization but there should be no serious objections to use of these recommended units.

Since there are so many biological variables to be dealt with in this type of work, and since clinical laboratory methodology is one of the few areas which may be reasonably controlled, it behooves us all to begin solving the monumental problem of providing comparable data by starting with the same laboratory methods and terms.

TABLE I
STABILITY OF SERUM OR PLASMA SAMPLES

<u>COMPONENT</u>	<u>REFRIGERATED</u>	<u>FROZEN</u>
Protein, Total	4 weeks	-----
Protein, Electro- phoresis	5 days	-----
Urea Nitrogen	3 days	6 months
Calcium	3 days	6 months
Sodium	3 days	6 months
Potassium	3 days	6 months
Phosphatases	3 days	-----
LDH, Electro- phoresis	-----	-----
Glucose	-----	-----
Phosphate, Inorganic	3 days	6 months
Transaminases	3 days	-----
Chlorides	3 days	6 months
Bilirubin	-----	-----
Cholesterol	-----	-----
Glutathione	-----	-----
Methemoglobin	-----	-----
BSP	-----	-----

TABLE II

<u>COMPONENT</u>	<u>MEASUREMENT METHOD</u>	<u>REACTION PRINCIPLES</u>
Bilirubin	Colorimetric	Diazo Reagent
Calcium	"	Chloranilic Acid/ EDTA
Chloride	Titration	Mercuric Nitrate
Cholesterol	Colorimetric	Ferric Chloride
Glucose	"	Glucose Oxidase
Glutathione	"	5, 5'-Dithiobis-(2- Nitro-Benzoic Acid)
Hemoglobin	"	Potassium Ferri- Cyanide, A. At 540
M-HGB	"	Na Cyanide, A. Diff.
LDH, Isoenzymes	Electrophoretic	Discontinuous-Gel
Phosphatases	Colorimetric	Phenolphthalein Phosphate
Phosphate, Inorganic	"	p-Methylamine- Phenol Sulfate (ELON)
Protein, Total	"	Biuret
Protein, Fractionation	Electrophoretic	Discontinuous-Gel Cellulose Acetate
SGOT	Colorimetric	Oxalacetate-Hydra- zone
SGPT	"	Pyruvate Hydrazone
Urea N	"	Urease-Nessler
Sodium	Flame Photometry	-----
Potassium	"	-----
BSP	Colorimetric	Dose = 10 MG/KG Sample Collect = 15 Minutes

TABLE III
PRECISION OF METHODS

<u>COMPONENT</u>	<u>REPRODUCIBILITY</u>
Bilirubin	$\pm 5\%$ (over 3 mg%)
BSP	$\pm 3\%$ (over 20% Retention)
Calcium	$\pm 2\%$
Chloride	$\pm 2\%$
Cholesterol	$\pm 5\%$
Glucose	$\pm 5\%$
Glutathione	$\pm 5\%$
Hemoglobin	$\pm 3\%$
Methemoglobin	$\pm 5\%$
Phosphatases	$\pm 2\%$ (over 10 units) $\pm 5\%$ (except in very low normal range)
Phosphate, Inorganic	$\pm 4\%$
Protein, Total	$\pm 3\%$
Protein, Electro- phoresis	$\pm 10\%$
Transaminases	$\pm 2\%$
Urea Nitrogen	$\pm 5\%$
Sodium	$\pm 2\%$
Potassium	$\pm 2\%$

TABLE IV
SERUM VOLUMES REQUIRED

<u>COMPONENT</u>	<u>SAMPLE SIZE REQUIRED (ML)</u>
Bilirubin	0.040-0.800
Calcium	0.020-1.000
Chloride	0.010-1.000
Cholesterol	0.200-1.000
Glucose	0.020-1.000
+Glutathione	0.200
+Hemoglobin	0.200
+Methemoglobin	0.100-0.200
LDH, Electrophoresis	0.005-0.015
Phosphatases	0.400
Phosphate, Inorganic	0.025-1.000
Protein, Total	0.020-1.000
Protein, Electrophoresis	0.010-0.020
Transaminases	0.200
Urea Nitrogen	0.010-0.500
Sodium	0.100
Potassium	0.100
BSP	1.000

Approximate Volume Needed for Ultramicro-Micro
Techniques = 2.50 ML

Approximate Volume Needed for Macro-Micro
Techniques = 9.50 ML

+Whole Blood

TABLE V
STANDARDIZATION OF REPORTING UNITS

<u>COMPONENT</u>	<u>RECOMMENDED UNITS</u>
BSP	% Retention
Bilirubin	Milligrams / 100 ml blood (mg %)
Calcium	Milliequivalents / Liter (meq/l)
Chloride	Meq/l, as Sodium Chloride
Cholesterol	Mg %
Glucose	Mg %
Glutathione	Mg %
Hemoglobin	Gram %
Methemoglobin	% of Total Hemoglobin
LDH, Isoenzymes	% of Total for each band
Phosphatases	Klein-Babson-Read Units (KBR Units)
Phosphate, Inorganic	Mg %
Protein, Total	Gram %
Protein, Fractionation	% of Total for each Fraction
Transaminases	Reitman-Frankel or Sigma-Frankel
Urea Nitrogen	Mg %
Sodium	Meq/l
Potassium	Meq/l

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EXPERIMENTAL BIOLOGICAL AND BIOCHEMICAL PARAMETERS

By

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The published papers of the 1963 Symposium on Toxicology in Closed Ecological Systems contained several requests and rather strong justifications by persons experienced in environmental toxicology for establishing criteria which might be used, not only to minimize the cost of the kinds of investigations we are considering here, but also to serve as a basis for comparison of data between laboratories ⁽¹⁾. However, none of these articles contained a suggested list of tests which, in the author's experience, might serve as a point of departure for establishing such a set of minimum criteria.

This is understandable, for on practical grounds it is virtually impossible to arrive at a set of measurements that will be uniformly accepted for evaluating the functional integrity of all of the organ or metabolic systems that might be adversely affected by the mixture of contaminants generated in an inhabited confined space. Thus, I interpret my function as being one of suggesting specific things to do which may at least partially satisfy these requirements and which, at the same time, will give leads to the work which must follow to more precisely define the underlying causes of the disturbed physiology that might be observed.

As an operating philosophy, I think the statement previously made by Captain Siegel ⁽¹⁾ can hardly be improved upon. In a condensed form, he said that the successful accomplishment of the assigned mission of a crew of a confined space depends on the ability of this crew to perform tasks with the highest degree of efficiency, and, above all, to survive without irreversible disease or organ damage. Everyone is aware of the uncertainties involved in extrapolating data from animals for use in man, but, at the same time, all have recognized the value of, and indeed the indispensable place of research using non-human subjects. Therefore, what I say today will be slanted to observations that can be made on laboratory animals. I have tried to select (a) tests that may be conducted without the active participation of the subject; (b) tests that do not require general anesthesia or other major manipulations that may complicate the overall exposure experiment; and (c) tests that are sufficiently routine that they may be performed by conscientious clinicians and well-trained technicians in a modern, well-equipped laboratory. As a matter of fact, virtually all of these procedures have already been used to advantage in animals and men in studies of atmospheric contamination ^(1, 2, 3).

To be sure, most of the things I will propose first are subjective determinations. Nevertheless, they are important, since changes in these simple items of observation often signal the development of pathologic physiology. I realize that each of us in this room is acutely aware of the value of a good medical history and an exacting physical examination; but I often have the feeling that, as soon as attention is turned from man to the experimental animal, we suddenly lose confidence and require all sorts of quantitative measurements to make us aware of what we see. So, I think it is worth emphasizing that much information can be gained from a reasonably simple physical examination.

For the examination to be of optimal value, the clinician should have studied the animals sufficiently long prior to the exposure for him to adjust his sights to the norm, or the baseline, against which he will make future comparisons.

The examinations should be made near the same time of day (I suggest they be made around mid-morning to allow time for the animals to have become accustomed to the usual daily increase in human activity). They should be regularly scheduled and frequent. Daily examinations may be in order at the beginning of the experiment; but, depending on the findings, they may be decreased to every second day or so as the experiment progresses. They should be thorough and unhurried; and, as a minimum, should include the following things:

1. Observe the General Behavior of the Animals. Particularly note such things as a change of "personality", or developing malaise or lethargy, or hyper-excitability. Also, note the muscular coordination and equilibrium of the animal, as well as the simple reflexes such as the blink reflex, the palpebral reflex and the placing reflex. All of these give some clues to the functional integrity of the C. N. S. The examination of general behavior, with regard to the ability of the animal to carry out specific tasks, lies in the realm of the experimental psychologist and will be discussed later in this Conference by Major Reynolds.

- 1a. In man, this facet of the toxicological study may be one of the most discriminating in detecting subtle changes induced by the combined influences of the confined space, the work schedule, and the atmospheric environment.

2. Observe Unusual Pupillary Size and Pupillary Reactions which, along with other portions of the examination, are indicative of autonomic function. While examining the eye, observe the sclera for jaundice, and, in the case of albino animals, take advantage of their non-pigmented iris to note possible cyanosis.

- 2a. In man, the examination of the eye can be refined to give quantitative data on changes in vision, accommodation and pupillary responsiveness.

3. Note the Condition of the Conjunctivae, of the Skin and of the Oral Mucous Membranes. Not only are these observations informative with respect to the contaminants possibly being contact irritants, but also should skin or buccal infections occur, they may indicate a possible derangement in the mechanisms which normally protect against surface growth or invasion of microorganisms ⁽⁴⁾. It should be remembered that the pliability and texture of the skin and subcutaneous tissues change markedly in dehydration and in edema.

4. Carefully Observe the Breathing Rate, the Character of the Breathing and the Chest Sounds. The portions of the chest used for stethoscopic examination should be clipped so the hair will not crackle under the stethoscope and obscure fine, moist, pulmonary rales. Of course, electrocardiography can be done. If used, one should be very cautious in attaching significance to moderate changes in the amplitude of the recorded complexes. Nevertheless, the ECG is useful in confirming the development of conduction defects.

4a. When a cooperating subject such as man is available, the examination of the respiratory system can be improved by carefully conducting such simple pulmonary function tests as the timed vital capacity, maximum breathing capacity and single breath nitrogen washout.

4b. Also, in man, in which ECG technology and interpretation is considerably more advanced, this procedure may be used to greater advantage.

5. Pulse Rate, Rhythmicity and Quality, as well as Heart Sounds, should be noted.

6. Rectal Temperature is an important datum and should be recorded.

7. Quantity of Intake of Feed and Water, both Quantity and Character of Feces and Urine, and Body Weight should be closely followed and recorded.

7a. In man these simple observations may be supplemented by a determination of the basic metabolic rate.

So, this is the first part of a suggested list of minimum observations to consider. It is not sophisticated in the sense that it requires a great deal of equipment, but it will provide at least an indication of disturbances occurring (a) in portions of the nervous system; (b) in the respiratory system; (c) in the cardiovascular and urinary systems -- particularly if the disturbances are leading to edema or dehydration; and, (d) in the various systems involved in ingestion, digestion, absorption, utilization of nutrients and in excretion of wastes.

The criteria used for interpreting these observations in laboratory animals reside largely within the experience of the clinician. Normal values

for a few of the items have been published. The "Handbook of Biological Data" and the "Biology Data Handbook" are handy sources of this information and probably should be accepted as our most valid general references for normal criteria (5, 6).

Naturally, we should not totally rely upon the physical examination, for it leaves too much retrievable data untouched. The big problem is selecting a manageable group of meaningful, supporting and supplementary laboratory examinations from the literally hundreds that could be done.

It is important to select specimens for laboratory analysis whose collection imposes as little additional stress as possible on the experimental subject. The four we usually think of are urine, feces, venous blood and saliva.

Insofar as the minimum tests for animal experiments are concerned, we may rule out saliva as a suitable test material, although this fluid certainly has potential usefulness for investigations in man. Both urine and feces may be considered, but it is frequently difficult to collect uncontaminated specimens without using procedures that may introduce complications to the overall experiment. On the other hand, specimens of venous blood are usually easy to obtain and can be used to great advantage (7). Therefore, most of the recommended laboratory procedures will relate to this tissue. Naturally, the frequency of conducting laboratory examinations must be less than the frequency recommended for the physical examinations. Two or three control samples collected at weekly intervals should be obtained prior to initiating the exposure to the potential toxicant. Weekly sampling during the exposure should provide sufficiently close surveillance.

1. The packed red cell volume gives an indication of hemolysis, anemia or hemoconcentration. The high-speed "microhematocrit" technique is in general use and gives very reproducible results when a uniform time of 5 minutes is used for the centrifugation (8).

2. Supplementary information on erythropoiesis and release of red blood cells into the circulation can be obtained from reticulocyte counts (9). However, since reticulocytes ordinarily comprise such a small fraction of the total RBC's, the error in these counts is large, and at least 2,000 cells should be counted (3,000 - 5,000 are better). If reticulocyte counts are made, the technician should also observe the slide for the "platelets" (10). An actual platelet count is not indicated, but an appreciable deviation from normal will be apparent to an experienced technician.

3. Hemoglobin concentration should be determined. Selecting the proper technique to use always seems to create controversy. This is because of the possibility of chemicals forming complexes with hemoglobin which might interfere with the light absorption at the particular portion of the spectrum selected for measurement. This possibility assumes real importance in the

current discussions since the experiment assumes that the subjects will be exposed to a variety of chemical contaminants. Therefore, I suggest we merely record a complete absorption spectrum from about 450 to 650 $m\mu$. The blood sample may be diluted in distilled water with a little saponin added. Also, a spectrum should be recorded of a similar dilution of the blood in Drabkins' Solution. Both of these spectra may be recorded on the same chart and will allow evaluation of the types and proportions of the various hemoglobins formed during the experiment (11, 12).

4. The leucocytes offer an important tool for evaluating a potentially hazardous environment. They not only reflect the metabolic integrity of the bone marrow and lymphoid tissues, but also give an indication of many infectious or allergic processes that may be developing. Both total and differential counts should be made. At least 200 cells should be counted for the differential.

5. Ordinary chemical determinations on serum offer so many possibilities for gathering potentially useful information that one is hard put to make appropriate selections (13, 14). It should be understood that all analyses be conducted in at least duplicate. Micromethods are in order (15, 16).

5a. The total concentration and concentration pattern of serum proteins (albumin, α_1 , α_2 , β and γ globulins) should be determined since these give an indication of one synthesis function of the liver and of lymphoid elements. For determining serum protein pattern, electrophoresis is the technique most commonly used. Since the absolute quantities of each of the protein fractions are slightly different for each of the several electrophoretic procedures, a basis for comparison between laboratories may be established by concurrent analysis of a certified standard.

5b. Serum bilirubin (direct and total) not only gives information that may indicate the type of anemia that might have been observed by the hematocrit; but in addition, it is an indication of the ability of the liver to carry out one of its many conjugation and excretory functions (17). In this context, urine and fecal urobilinogen determinations are not included as one of the recommended minimum tests because of the difficulty in routinely obtaining suitable samples. Bromsulphthalein excretion is not included for two reasons. First, it requires introducing a foreign substance, albeit presumably a nontoxic one, into the experimental subject. Second, the calculation of the rate of excretion depends on an estimate of the volume of dilution of the BSP. The volume of dilution is usually taken as an assumed fraction of the body weight. However, there is no a priori reason to believe that this fractional relationship will hold in our experimental subjects. To adequately define the volume of dilution, a second and more persistent foreign material must also be introduced into the animal. Therefore, I feel that assay of liver function by using endogenous materials such as bilirubin is to be preferred.

5c. Elevated serum creatinine concentration is an indicator of early kidney damage and should be measured.

5d. The pH and electrolytes of anaerobically collected and separated serum or plasma provide valuable indexes of the malfunction of the renal tubules and of the lungs, as well as of several endocrine systems. Although arterial blood provides the superior samples, particularly with respect to pH, bicarbonate and chloride, I think venous samples will meet our current requirements. Major deviations from normal can be detected and may be more closely investigated as a separate set of experiments.

6. Serum enzymes are enjoying increasing popularity as diagnostically useful tools. Here again one is beset with numerous possible choices. None of the enzymes I know of is sufficiently tissue-specific to allow unequivocal identification of the organ from which it is released into the plasma. Nevertheless, their appearance in greater than normal concentrations allows a reasonable conclusion that cellular membranes are being damaged, or that there is an abnormally large quantity of the enzyme being synthesized, or that normal pathways of degradation are inhibited. Any of these events should be of interest to us in the evaluation of the biological effect of atmospheric contaminants. As a minimum, I suggest determining one enzyme that uses an amino acid as a substrate (Serum Glutamic Oxaloacetic Transaminase), one enzyme that uses a fatty acid as a substrate (Serum Lactic Dehydrogenase), and one enzyme that uses a carbohydrate as a substrate (Serum Fructose - 1, 6-diphosphate Aldolase) (18, 19).

Lastly, I recommend for your consideration periodic physical and laboratory examinations of representative numbers of these animals for an extended time following the termination of the exposure.

IN SUMMARY

1. One should not overlook the value of a good physical examination repeated frequently.
2. Blood is probably the laboratory specimen of choice.
3. The recommended laboratory procedures are these:
 - a. Microhematocrit.
 - b. Reticulocyte count and observation of platelets (?).
 - c. Hemoglobin spectrum in water and Drabkins' Solution.
 - d. Total and differential leucocyte count.
 - e. Total plasma protein and proportional protein fractions.
 - f. Plasma or serum creatinine.
 - g. Total and direct serum bilirubin.
 - h. Plasma or serum pH and electrolytes.
 - i. Serum enzymes - GOT, LD, Aldolase.

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GROSS AND HISTOPATHOLOGICAL EVALUATION

By

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Thank you, Mr. Chairman. Ladies and Gentlemen. In his letter inviting me to take part in this session, Dr. Thomas indicated that he hoped to maintain a workshop atmosphere and a free exchange of ideas. I am happy to participate in such a workshop and I surely hope that a workshop atmosphere will prevail and there will be a free exchange of ideas. I, for one, hope to learn a great deal from participation in this Conference. In fact, Dr. Thomas, I have learned a great deal already.

A subtitle for my remarks today might well be "The Significance of the Necropsy" -- or in other words, what part does gross and microscopic examination play in laboratory studies such as we are discussing today, and, particularly, what minimal criteria must be met by investigators in this field so that the results will be valid and some comparison may be made from laboratory to laboratory?

At the start of any study, first we must closely define our real objective and then design an adequate experiment to attain that goal.

For instance, we should consider the type of study. Is it a range-finding study, or a study to determine the lethal concentration produced by a definite set of conditions, within a definite period of time? Is this a study directed particularly toward determining the target organs, including the nature of the toxic response and its seriousness in relation to health? Is this a study to determine the reversibility of pathological changes that may occur, or is this a study to determine the pathological changes that may occur following exposure? Is this a study to determine the "no-effect" level under given conditions? Or is this a full-blown toxicological study on a particular contaminant to learn as much as possible concerning its toxic action, including absorption, distribution and excretion of the toxicant itself and of its metabolic products?

Now, probably, we are someplace in between -- a range-finding study on the one hand and a full-blown toxicological research program on the other; but, in any event, one of our major objectives certainly will be to obtain sufficient toxicological data in order that this may serve as a basis for the assessment of the hazards to health under certain prescribed conditions.

However, let me repeat, the objective must be clearly defined and then the experiment so designed that we reach that objective.

In designing the experiment, it is very important that all professional people concerned with the experiment be included on the team. In other words, all professional people must be in on the planning of the experiment, and I point out particularly that I believe this applies to the pathologist and the statistician just as it does to the toxicologist, the biochemist, the pharmacologist, the psychopharmacologist, and all the rest of the people on the team.

Furthermore, in a study of this nature, a team effort must continue throughout the experiment. The pathologist, the statistician and all those others concerned, must play an active part and this means that throughout the course of the experiments, there must be an exchange of ideas between the people, a free exchange of suggestions, ample critical evaluation, constructive criticism and that they must work together toward the objective.

Many of the previous speakers this morning have covered a great deal concerning this design. Dr. Thomas has discussed the pertinent considerations of the chamber design. We've had a discussion of the production of the contaminants and analytical techniques concerned. Particularly I was especially interested in Dr. Innes' remarks. Frankly, I would like to relinquish my time and let him discuss dogs and swine and perhaps other species, because the matter of selection of animals for this type of work is extremely important to us and very often we have not given enough thought to it in the past. Let me merely reiterate here that it is important to consider the animal species and strain, the sex, the age, diet, caging, the actual environment, whether it's an air-conditioned laboratory or not, the light intensity, the darkness, the heat, the temperature, humidity control -- all of those things. In addition, even the ordinary matter of the laboratory environment, the routine handling of animals, and the day by day processes that go on in the laboratory. Then, of course, the exercise of the larger animals. All of these do play a significant part and many times it's such factors as these, which we do not often think about, that help to explain the apparent inconsistencies that do occur between laboratories.

I realize some of the things I'm saying are repeating, but perhaps they deserve repeating for emphasis. In matters of design in experiments like this, we cannot give too much attention to adequate control. This means concomitant controls maintained as nearly as possible in every respect identical to the caring for the exposed animals. This is especially true in the case of the larger animals, and when we get to some of the parameters that Colonel Reeves talked about, we must get baseline values on these. Oftentimes these larger animals can serve as their own best controls.

The type of baseline parameters that Colonel Reeves talked about will help us a great deal in ruling out abnormal and diseased animals prior to starting the experiment.

In this connection, in my work on vapor exposure or intratracheal injection, we found X-rays on rats that helped to some extent in ruling out animals with obvious murine pneumonia. Dr. Innes was certainly right, this murine pneumonia situation in the ordinary colony is a very, very real concern.

Now, I would like to turn to the actual planning for the autopsy. I believe the pathologist should be fully aware of the pertinent information that has been obtained during the course of the experiment. He must know about this, and he must have the cooperation of the clinical men that made the observation, the biochemist that carried out the studies, and so on. He must go into the autopsy with his eyes open, because surely there are many factors that will influence what the pathologist does in autopsy and examination of tissue. It is important that he knows the mortality in the group; he knows about the growth; the actual body weight loss and gain; food and water consumption; the hematological picture that has been determined will influence his decision as to how much bone marrow study should be carried out; the biochemical findings; and surely any results of the behavioral studies. In addition, if there have been earlier studies in which "target-organs" or "target-systems" have been pointed out, he should be aware of this and design his autopsy accordingly. And, obviously, he should be aware of pertinent literature on the compound under study or closely related materials.

Before actually performing the autopsy, I think first we need a terminal examination of the living animal. This is really finishing up the work that you started and carrying it on, until the time of autopsy.

Some of these you can do on a dog very easily: pulse, respiration, temperature, body weight, ordinary neurological examination, eye examination, and, of course, X-rays, EKG's and respiratory function tests can also be carried out. This, I think, will help a great deal in designing what is to be done at autopsies. Even in the smaller animals, a well-trained observer can make many worthwhile observations. These include just the general appearance of the animal, such things as cyanosis, dyspnea, condition of the coat, staining of the fur, posture, movements, CNS depression, paralysis, righting reflex and other neurological signs, tremors, ataxia, muscular weakness, etc.

Now, I would emphasize that when these observations have been made, an adequate written record must be kept. I think this is where a lot of us fall down, whether we be clinicians, whether we be biochemists, whether we be pathologists, even statisticians, I suspect that we fail to make the

written observation at the time. Obviously, this information obtained from the living animal will influence a great deal the work carried out by the pathologist's autopsy.

My very strong feeling is that the actual autopsy should be done by the pathologist, at least under his close observation. I feel the man who will actually be looking at the slide should also see the gross specimens. Now, here's a point of argument, I'm sure, but I do believe there is much to be gained by this procedure.

A great deal can be gained by careful, systematically performed necropsy. Here again, the consistency and technique is very important. Consistency first and foremost in the matter of the method of killing the animal. Is the animal starved or not starved prior to killing? Then the method of killing the animal, the method of bleeding the animal and then the matter of the systematic dissection. Again it is imperative that the control animal be treated in every respect the same as an exposed animal. I know I'm belaboring this point, but I do feel it is very important.

The dissection should be carried out systematically and gross lesions described. Here again, I think we fail. The location, the severity, the extent and the size of the lesion should be noted and graded. Certainly most laboratories have developed their own "keys" for descriptive purposes and for grading, which, of course, helps in the record.

In the course of the type of experiment we are discussing today, continuous inhalation work, surely we must give attention to the respiratory system. At least, we must look at the condition of the nose, mouth, sinuses, turbinates, trachea, the lung, all of the regional lymph nodes, the condition of the cavity itself, the heart and so on. I think this is obvious. Sometimes we don't do this, but most certainly we should. The tying-off of the trachea prior to opening of the cavity will prevent deflation of the lungs, and very often this helps a great deal in the observation of the lung and the future work with the lung.

In addition to the respiratory system, we obviously should consider the abdominal cavity, the kidneys, the liver, the spleen, the urinary bladder, adrenals, gonads, mesenteric lymph nodes and the G.I. tract. Then, very often, and I'm sure we could at least grossly consider the skin, the subcutaneous tissue, the muscle and bone and all other structures. Something we always should watch for is tumors, noting their location, size and the occurrence as nearly as we can. Ordinarily in the larger animals, the brain and the cord are examined grossly, and gross observations recorded.

We like to maintain a permanent camera setup so that one can record photographically the gross findings. This has been extremely helpful to us in lung work. We also find that gross mounts are very helpful for demonstration purposes.

The obtaining of organ weight is also a very important part of an autopsy. Consistency is most important, even the matter of care in dissection, the matter of the procedure used in washing, draining, blotting and weighing is by no means consistent from laboratory to laboratory. Most certainly a common variable is simply the time lag between blotting or draining, whatever one does, and the actual weighing. This can make a big difference in the case of some tissues. Here, again, the cardinal thing, I think, is to treat the controls exactly as you treat the experimental animal.

The organs commonly weighed, and I think all of us do about the same here, and I'm not sure we're absolutely right, but we do weigh the lungs, heart, liver, kidney, testes and spleen regularly. Much less frequently such organs as brain and adrenals, and rarely other organs such as the thyroid, pituitary, even the gall bladder, urinary bladder, ovaries, and occasionally the G.I. tract.

We commonly express values, of course, in either absolute values, in grams or grams per kilogram, (or G/100 G) body weight, much less frequently, although sometimes it is important, on the basis of G/100 G brain weight, and rarely, G/100 G heart weight or G/100 G testes weight. I would say here in the case of organ weight that it is possible that recording can be done by electronic data processing procedures. We are using them in our laboratory. They can be used in such a way that we can get a listing of the organs by group and get an average, a standard deviation calculation, and statistical comparisons.

We are using electronic data processing. We feel it is practical. I'm anxious this afternoon to hear more about various statistical methods that may be used, including data handling, data processing and retrieval. But it has proven helpful in the case of organ weight and body weights in our laboratory.

Now, let's turn to the matter of histopathological examination itself. The obvious thing that comes to mind is how do you prepare the tissues and what tissues do you examine? I think the most common procedure used in the preparation is neutral formalin fixation, paraffin embedding and hematoxylin and eosin staining. Only rarely are other stains used routinely. The use of other stains may well be dictated by either the gross examination or the findings from the H and E stains, or from the other work during the experiment. Now, this is a very large laboratory load, as each of you know. We find that using the Auto-Technicon helps a great deal as far as dehydration, embedding and staining are concerned. By placing several sections, particularly in the smaller animals, in one block at least you cut down on the numerical number of slides that have to be cut, handled, labeled, looked at and filed. This, perhaps, helps a bit.

Now, so far as the tissues to be examined, I'd say there's only one real rule here, the first rule of thumb, and that is, for goodness sake, every

lesion that is observed grossly -- look at it microscopically. This, again, emphasizes the importance of the gross examination.

If there are any indications from the literature of "target organs" or actual experiments with related materials which have already been carried out; or, conversely, if you have already information which would definitely rule out the necessity of studying certain systems, then look at least, at those organs that have shown gross pathology or are indicated by the literature.

In evaluating an experiment in which several levels of concentration have been used, you may be able to save some time by looking at the tissues at the top level and then working down, until you reach the "no-effect" level.

In this work, I would emphasize the importance of concurrent controls, that is animals carried under identical conditions, because, when you get right down to it, the histopathological examination in these studies actually comes down to an exercise in "comparative pathology" where you do compare carefully the findings on the experimental animals with the findings of the controls, and you judge differences in kind, in incidence and in degree.

Now, what organs should be sectioned? Well, certainly in animals that have received a continuous inhalation exposure, you want to look closely at the respiratory system and, without any doubt, one should have the lungs, the heart, the trachea, the regional lymph nodes, and anything else that, in the opinion of the pathologist, one should have. We have found, of course, in the case of the small animal like the rat, that the trachea, thyroid, parathyroid and esophagus can be obtained in one section. In addition, liver, kidney, adrenal, pancreas, mesenteric lymph nodes, urinary bladder, testes, the ovaries or the bone marrow surely are minimum.

Less frequently, when you can go practically across the board, the G.I. tract very commonly is taken. Very often it is productive. The skeletal muscle, peripheral nerve, bone, prostate or uterus, skin, eye, in many cases the thymus, are also taken in the rat. And, of course, all tumors should be looked at microscopically.

And how about the central nervous system? I know I'm sticking my neck out here, but I would say generally for the type of experiment we are talking about -- in the absence of any gross abnormality on the brain or cord, and in the absence of any significant findings in the neurological examination and behavioral studies -- that only a few sections of the brain and the cord need be prepared for H and E staining. This is due in large part to the fact that the interpretation of microscopic findings in the brain and cord is extremely difficult and usually requires examination by a well-trained, experienced neuropathologist familiar with the particular animal species you're studying. For a real good histopathological examination of the brain or cord, one needs special fixation, perhaps some infusion techniques and special staining. So

this is a specialty field. I think we should keep our eyes open where we truly have a problem in a specialty field, and, for goodness sake, let's get a well-trained neuropathologist to look at the slides.

You will note throughout this discussion that I have emphasized the pathologist should have the benefit of the full knowledge of the experimental design and the objectives to be accomplished by this experiment. He should have complete knowledge concerning the prior history of the animal at the time it reaches him for autopsy. Then, what is the place for "blind" examination of pathology tissue? I think there's a real place for "blind" examinations, particularly in "border-line" situations, particularly where you are concerned in the grading of lesions; and I think that very often a consulting pathologist that does tackle a job blind, studying randomized sections, can be of extreme help to you. Now there may be other places, but this certainly is one place.

I will not discuss the techniques and uses of histochemistry or electron-microscopy. I realize these are most interesting areas of research and I have no doubt but that in the future they will be very valuable and will have great importance in work of this nature. However, I believe, except in cases where there are definite indications of the need for such studies, that they should not be undertaken on a routine basis in studies of this nature.

Now, turning to the tabulation, handling and evaluation of data, I do believe the proper coding and programming can be worked out so that electronic data processing methods can be used. I have already mentioned that we are doing this in the case of organ weight and I believe that with systematizing of nomenclature, data processing procedures can be used in handling and evaluation of all data.

I'd say specifically it can handle the observations on the living animals, including hematological, biochemical parameters that have been discussed -- clinical data. It can handle observations made at the time of autopsy and certainly the histopathological findings. Very recently the American College of Pathologists has published a compilation of "Systematized Nomenclature of Pathology", which is designed and directed specifically toward this objective.

Now, how about our minimal requirements that must be met, specifically so far as gross and microscopic examinations are concerned? In actual practice, what are the most efficient criteria that can be used? I think it's first obvious that we cannot so stereotype these studies that they become nothing more than "testing" procedures. The investigators must be in a position to carry out scientifically sound studies with scientifically sound observations by qualified personnel.

Nevertheless, all of us realize that, of necessity, in any given study we are forced to pick and choose the most efficient criteria for determining the significant effects which are consistent with the objective of the experiment that we are undertaking.

In this connection, the publication by Rowe and Wolf from our laboratory and Weil and Smyth of the Union Carbide Chemical Hygiene Fellowship at Mellon Institute, I think is quite pertinent. This paper, published in 1959, summarizes the results of more than 400 studies carried out in these two laboratories over the past 25 years. The results were tabulated from studies involving repeated administrations, either by inhalation or by ingestion, for periods of time ranging from one month to two years.

It was found that "if only the five criteria," (and these five criteria are growth, liver and kidney weight, and liver and kidney histopathology) -- "if only those five had been studied, the lowest dosage level found to cause any effect would have been detected in 96 per cent of the cases. Now it is readily apparent that these five criteria as a group were far more efficient in detecting first signs of adverse effects than most of the numerous other studies quite commonly included in toxicological investigations."

These authors conclude that "this does not mean that only those criteria showing a high degree of efficiency in the series of investigations under study should be used, but it certainly suggests that they should be basic to any toxicological study designed to determine a threshold limit for repeated exposure."

In addition to the above five criteria, for continuous inhalation exposure studies, I would certainly urge that lung weight and lung gross and histopathology be used. Also, I believe the heart and testes, spleen and adrenal weight and pathology should be recorded. If indicated from peripheral hematology, bone marrow should be included.

In summary, these are the minimum requirements. At the very least, every lesion observed grossly should be looked at microscopically. And, furthermore, leads from the literature and leads from related materials should not be overlooked. Above all, I believe that the pathologist must be free to take additional tissues and to carry out additional studies that he believes to be necessary to reach the objective.

In conclusion, and Mr. Chairman, I think this could well be a conclusion for the whole session this morning, I'd like to quote Dr. Lehman. I've quoted this many times and I believe that it is very appropriate.

"There can be no formula, ratio or substitute for the mature and considered judgment of men fitted by experience and training for making the final recommendation. In the last analysis, the caliber of the men conducting the experiment and making the evaluations determine the usefulness of animal toxicity data."

SESSION II

STATISTICAL METHODS OF EVALUATION AND INTERPRETATION
OF EXPOSURE DATA

Chairman

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SESSION II

Opening Remarks Dr. H. C. Hodge, Chairman

During Session I we had a lot of questions, and these questions had to do with very fundamental aspects, like "what is the point of the investigation and is it being approached in an efficient way". And some of these questions that really haunt us, like "are there subtle effects that are unnoted", are enough to give any good toxicologist a nightmare just to bring it into his consciousness. We are admonished, and I think wisely, not ever to think in terms of rigid, frozen, stereotyped programs. The thing that appealed to me, though, very much was how many times people got around to the idea that there had to be a common language, and maybe this is one of the secrets that we'll have to think about. Perhaps we should work toward, as a part of our establishing good understanding, which we were advised to do at the very beginning, getting a common, agreed on, defined vocabulary, a glossary. This morning, then, set the stage for the problems of results and we can ask a lot of questions about results too. The first question that always pops into my mind, can you repeat it? The second -- you want to know how accurate it is, what ballpark? Are we talking about 10% or 50% or 1%, and, of course, what are the effects? The serious-minded man says "by what mechanisms", and the next fellow says, "what do they mean?". Then we come to the real sticky one, how do these apply to man?

Session II is really two parts, and if you will permit me, I will delete the and and substitute a semicolon and read this title as "Statistical Methods of Evaluation; Interpretation of Exposure Data". We're going to be looking at this field, at these questions, in a number of ways.

STATISTICAL METHODS IN TOXICOLOGICAL RESEARCH

By

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Introduction:

The statistical theory has been applied to toxicology in many different contexts. This application has resulted in the development of the subject of Statistical bioassay and has in turn led to many new statistical techniques such as the well known stochastic approximation procedures (ref 1). There is continuing interest in problems of statistical bioassay as is evident from recent work in Bayesian bioassay (ref 2).

There are many other techniques of statistical inference which have been found useful in toxicology. Mention may be made of standard t and F tests of hypotheses, confidence intervals and mortality statistics (ref 3). The involved nature of multivariate statistical tests has not led to their widespread use in toxicology. However, we feel that the introduction of computers in research will make such techniques easily accessible. The recent developments in the non-parametric statistics also are bound to be useful in the study of toxicological problems. Methods of mortality statistics are too well known to require much discussion.

It is not possible here to discuss in detail the above techniques and their possible application in toxicological problems in general. In this paper, we shall try to discuss those statistical techniques which are specially relevant to the study of the effect of contaminated atmosphere on animals in confined spaces.

Some aspects of these problems are very important to scientists studying health problems in submarines as well, see for example Stark (ref 4), Schulte (ref 5) and Schaffer (ref 6).

Comparison of two populations:

Researchers are interested here in investigating the effect of certain environmental conditions on physiological changes in blood and tissues of various animals. Suppose we denote the blood variables by X_1, X_2, \dots, X_k ; there being k such variables like the number of red blood cells, number of

white blood cells, amount of sodium, potassium, calcium, albumin, total protein, neutrophils, etc. We, essentially, have for any time period of the experiment, observations on the vector

$$X = \begin{bmatrix} X_1 \\ X_2 \\ \vdots \\ X_k \end{bmatrix}$$

We shall consider the case of the pathology of the tissues in a similar manner later.

The components of X are easily noticed to be stochastically dependent. We assume therefore, that X has some kind of multivariate distribution. For simplicity, we shall assume that this distribution is multivariate normal, having a mean vector

$$\mu = \begin{bmatrix} \mu_1 \\ \mu_2 \\ \vdots \\ \mu_k \end{bmatrix}$$

and variance-covariance matrix

$$\Sigma = \begin{bmatrix} \sigma_{11} & \sigma_{12} & \cdot & \cdot & \cdot & \sigma_{1k} \\ \sigma_{21} & \sigma_{22} & \cdot & \cdot & \cdot & \sigma_{2k} \\ \cdot & \cdot & & & & \cdot \\ \cdot & \cdot & & & & \cdot \\ \cdot & \cdot & & & & \cdot \\ \sigma_{k1} & \sigma_{k2} & & & & \sigma_{kk} \end{bmatrix}$$

where σ_{ij} is the expected value of $(X_i - \mu_i)(X_j - \mu_j)$. That is, σ_{ij} is the covariance between X_i and X_j . The above facts may be summarized in the statement that X has $N(\mu, \Sigma)$.

In reviewing the literature on the applications of statistical techniques to toxicological problems, it has been noticed that assumptions of this type are not generally made by researchers. The multivariate analysis is used to the extent of calculating correlation coefficients which do give the tests

of hypotheses of dependence when normal assumptions are made. However, the application of multivariate statistical techniques so as to make various inferences such as testing equality of means, testing equality of variance-covariance matrices, problem of discrimination among several populations, etc., are yet to find a widespread use in toxicological research, see for example Tiedeman, Kogan and Wantman (ref 3), Weil (ref 7), Jackson (ref 8). We shall indicate some useful multivariate techniques in what follows.

Suppose we are interested in knowing whether the means of the blood vector remain the same under the two experimental conditions, say, control and exposure. Let us denote the blood vector by Y in the case of exposure and let Y have $N(\nu, \Sigma)$. The object here is to test the hypothesis,

$$H_0 = \mu = \nu$$

$$H_1 = \mu \neq \nu$$

under the assumptions of unknown but equal Σ 's. Suppose X^1, X^2, \dots, X^n are n observations on the vector X and Y^1, Y^2, \dots, Y^m are m observations on the vector Y . Let

$$S = \frac{1}{m+n-2} \left[\sum_{\alpha=1}^n (X^\alpha - \bar{X})(X^\alpha - \bar{X})' + \sum_{\beta=1}^m (Y^\beta - \bar{Y})(Y^\beta - \bar{Y})' \right]$$

where \bar{X} and \bar{Y} denote the vector of the means of observations on X 's and Y 's and A' denotes the transpose of the matrix A .

Then the test of the above hypothesis is based on the statistic

$$T^2 = \frac{mn}{m+n} (\bar{X} - \bar{Y})' S^{-1} (\bar{X} - \bar{Y})$$

which is a two-sample Hotelling's T^2 . The critical region is given by

$$T^2 \geq \frac{(n+m-2)k}{n+m-k-1} F_{k, n+m-k-1}(\alpha)$$

where $F_{k, n+m-k-1}(\alpha)$ is the $(1-\alpha)^{th}$ percentile of the central F with k and $n+m-k-1$ degrees of freedom. Alternatively we may say that the distribution of

$$\frac{T^2(m + n - k - 1)}{(m + n - 2) k}$$

is central F with k and m + n - k - 1 degrees of freedom under the null hypothesis. The details of this test are given by Anderson (ref 9).

For example, suppose that there are 20 observations during a control period and 4 observations during an experimental period on dogs, of the following variables

X_1 = potassium

X_2 = calcium

X_3 = protein

That is n = 20, m = 4, k = 3. Let the data be summarized by the following denoting by Y_1, Y_2, Y_3 the above quantities in the exposure period.

$$\begin{aligned}\bar{X} &= \begin{bmatrix} 5 \\ 4 \\ 3 \end{bmatrix} \\ \bar{Y} &= \begin{bmatrix} 5 \\ 5 \\ 4 \end{bmatrix} \\ S &= \frac{1}{20} \begin{bmatrix} 16 & 15 & 10 \\ 15 & 25 & 12 \\ 10 & 12 & 9 \end{bmatrix}\end{aligned}$$

The inverse of the matrix S is given by,

$$S^{-1} = \frac{22}{371} \begin{bmatrix} 81 & -15 & -70 \\ -15 & 44 & -42 \\ -70 & -42 & 175 \end{bmatrix}$$

We have,

$$\begin{aligned}T^2 &= \frac{80}{24} \times \frac{22}{371} \begin{bmatrix} 0 & -1 & -1 \end{bmatrix} \begin{bmatrix} 81 & -15 & -70 \\ -15 & 44 & -42 \\ -70 & -42 & 175 \end{bmatrix} \begin{bmatrix} 0 \\ -1 \\ -1 \end{bmatrix} \\ &= 26.68.\end{aligned}$$

Now $\frac{T^2}{22} \times \frac{20}{3} = 8.08$. This value is significant at 1% level. That is, the hypothesis H_0 is rejected at 1% level.

Case of many populations:

The test of the hypothesis of equality of mean vector of more than two multivariate normal populations having the same covariance matrix can be done by the techniques of multivariate analysis of variance. A considerable amount of literature exists now in this area, see for example, Smith, Gnandesikan and Hughes (ref 10), where many other references in this area are quoted. These authors give a computer program for model I of multivariate analysis of variance in addition to giving simplified steps to perform the analysis. Mention may be made of some graphical techniques developed by Wilk and Gnandesikan (ref 11) to deal with such multiresponse experimental data.

Comparison of pathology:

Many times it is required to compare treatments with respect to the pathology of certain organs of animals. Suppose that in an organ one has some way of rating pathology from no pathology to most severe pathology. This may be done with the help of ranks, say, 0, 1, 2, 3, 4. Let the probability with which these values occur be p_0, p_1, p_2, p_3 and p_4 respectively.

The probability of obtaining X_0, X_1, X_2, X_3 and X_4 cases in a sample of n observations with pathology 0, 1, 2, 3, 4 respectively, then the multinomial distribution

$$\frac{n!}{X_0!X_1!X_2!X_3!X_4!} \begin{matrix} X_0 & X_1 & X_2 & X_3 & X_4 \\ p_0 & p_1 & p_2 & p_3 & p_4 \end{matrix} .$$

If n is large, the above probabilities can be approximated by an appropriate multivariate normal distribution and techniques of usual multivariate analysis can be utilized for making inference. That is, for large n , the random vector

$$X = \begin{bmatrix} X_0 \\ X_1 \\ X_2 \\ X_3 \\ X_4 \end{bmatrix}$$

has multivariate normal distribution

$$\mu_0 = \begin{bmatrix} np_0 \\ np_1 \\ np_2 \\ np_3 \\ np_4 \end{bmatrix}$$

and

$$\Sigma_0 = \begin{bmatrix} np_0(1-p_0) & -np_0p_1 & \dots & -np_0p_4 \\ -np_0p_1 & np_1(1-p_1) & \dots & -np_1p_4 \\ \vdots & \vdots & \ddots & \vdots \\ -np_0p_4 & np_1p_4 & \dots & np_4(1-p_4) \end{bmatrix}$$

It should be remarked that multivariate tests and estimation procedures could be used here but to apply multivariate analysis of variance techniques, some further transformations will be needed.

Suppose we test the hypothesis that the exposure has produced more pathology as compared to control. Let the control group be represented by X_0, X_1, \dots, X_4 and the exposure group by Y_0, Y_1, \dots, Y_4 . We have the following table.

pathology Group	0	1	2	3	4	Total
control	X_0	X_1	X_2	X_3	X_4	n
exposure	Y_0	Y_1	Y_2	Y_3	Y_4	m

The usual tests for contingency tables based on chi-squared distribution will provide a simpler alternative to multivariate tests for testing the hypothesis of association. This technique works if the number of observations is large. In the case of a small number of observations, one must deal with exact methods to get significance levels.

If the number of groups to be compared is more than two, the chi-squared test still provides the answer. The general theory of these tests is easily accessible, see for example, Mood and Graybill (ref 12).

Related problems:

Studies on the effects of exposure of chemicals, gases and other environmental factors are found extensively in the literature, for a partial bibliography see Rustagi (ref 13). Most often the statistical techniques used are the simplest. The experimenter for example is satisfied by comparing two groups -- control and treatment groups with the help of t statistic. Sets of values with ranges and standard errors are provided many times by researchers.

Long-term studies involving chronic exposure to humans or animals are not few and they generate sufficient data to study the process more thoroughly.

Kehoe (ref 14) has conducted extensive experimentation on human subjects for the study of the effect of exposure to lead contamination in exposure chambers. Long series of data on lead in daily food intake, feces and urine are available. Sterling, Kehoe and Rustagi (ref 15) gave some mathematical models to study these effects. Recently some more deterministic and stochastic models have been proposed by Rustagi (ref 13) to obtain the behavior of the amount stored in the system when the behavior of intake and output are given. It has also been noticed that the trace substances in the intake and output of the human system follow a lognormal distribution, and many such cases have been discussed by Rustagi (ref 16). The additional information available in view of these mathematical models of the phenomenon under study, is of great importance to the experimenter as it reduces the number of observations required for an investigation and thus cuts the cost and time of the experiment.

Design problems:

The general routine of animal experimentation consists of providing the animals with experimental conditions but without any treatment so that they get acclimatized. This period is called pre-experimental so that the animals are essentially in pre-experimental control. These animals, then, can be further divided at random to be assigned to various experimental conditions including a control group. Since conditions of experiment are very precisely controlled in chambers or in cabin atmospheres, many experimental treatments can be compared with the same set of control animals if there is not much variation among the experimental animals.

The animals can act as their own control if certain responses over several time periods are observed. In many situations, if there is not much variability among the experimental animals, it is not necessary to have animals with their own control. A similar problem in connection with study of pain relief has been described by Meier and Free (ref 17).

Computer applications to statistical analysis:

The point of view that statistical work is less expensive and less time consuming than the collection of data has been expressed by many statisticians. This justification of trying sometimes alternative methods of analysis of data is further supported by the availability of large scale computers. Involved computations such as those required in multivariate statistical techniques and other linear as well as non-linear least-squares procedures are standard computer programs and are easily accessible to experimenters.

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PERFORMANCE MEASURES DURING EXPOSURE TO TOXIC ENVIRONMENTS

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INTRODUCTION

Historical and Philosophical Framework

Leake (ref 6), in his very fine review of the scientific status of pharmacology, tells us that as early as 1847 the pathologist, Virchow, recognized the implications for research that the characteristics of living material at different levels of organization have on the extrapolation of findings from one level to another. Today, Virchow's concept is an accepted fact and all of us are aware that biological organizational levels extend from macromolecules to ecological milieus.

Since analysis of the effect of a given chemical agent at the lowest levels of organizational structure does not always reveal the nature of relations between the parts of the intact organism and may actually destroy such relations (refs 1, 3, 5, 14), investigators should seek answers, wherever possible, from intact organisms. The reliability of the information gained, as a basis for clinical prediction, may be directly correlated with the increasing level of biological organization chosen for study. Eventually, as one pursues this type of thinking, it becomes quite clear that the "Whole is more than the sum of the parts" and that it is the overt behavior of a living intact organism to which the scientist must address himself. For overt behavior is perhaps the final question of survival potential, and the ability to effectively act upon and to interact with one's environment is the only means at a living organism's disposal to beget survival.

Physicians are well aware that the description of symptoms is not the same thing as diagnosing a disease, and how difficult it is at times to delineate between the symptomatic and fundamental aspects of various disorders. A similar circumstance also confronts the psychopharmacologist since he must continually search for the primary as well as secondary properties of behavioral impairment once an organism has been exposed to a particular toxic environment. But, from an objective, behavioristic point of view, it is unwise and unproductive in animal experimentation to speculate on what lies

within the animal. It may be motivated to do many things, but due to the influence of the toxic environment it simply is unable to translate its motivations into meaningful responses. This is analogous to the motor aphasic -- he may have many things he would like to say but he cannot say them (he can show us he has things to say because he can communicate them in writing) and, for purposes of acting effectively on his environment -- linguistically -- he is impaired. It is because of such observations that the thesis is advanced that the psychopharmacologist must limit his data to the quantifiable and verifiable aspects of an organism's functional existence.

Previous Efforts in Toxicological Research

The preceding research philosophy has guided the behavioral research accomplished by the 6571st Aeromedical Research Laboratory (ARL), Holloman Air Force Base, New Mexico, in support of the Toxic Hazards Branch, Aerospace Medical Research Laboratories (AMRL), Wright-Patterson Air Force Base, Ohio, since 1961. In that year, Reynolds *et al* initiated a series of experiments as a follow-up to the toxicological-physiological studies of Back, Thomas and others of the AMRL. This group has the major Department of Defense responsibility for investigating the biomedical effects of toxic hazards to which man may be exposed preparing for or during space flight. From 1961-63 the effects of 1-1, dimethylhydrazine (UDMH) on *Macaca irus* performance were studied (refs 9, 10). It was ascertained that 30 mg UDMH per kilogram of body weight is a critical exposure level, producing clinical signs of illness after two to three hours, a performance decrement or change after three to three and one-half hours, and recovery to the pre-experimental level between six and nine hours.

In early 1964 Reynolds *et al* (ref 11) studied the effect of decaborane on *Macaca mulatta* and *Macaca irus* operant behavior. Decaborane, $B_{10}H_{14}$, one of the boron hydrides, had, as a derivative of one of the high energy fuels, been extensively studied from pharmacological, toxicological and physiological points of view but no one had studied the effects on learned behavior (performance). Dosages of two and four milligrams per kilogram of body weight produced decrements in continuous motor behavior in all eight subjects and three-fourths of the subjects exhibited a decrement on the discrete tasks. In over half the subjects a performance decrement preceded clinical symptoms by a distinct time period, suggesting the importance of performance measures in any assessment of organismic functioning. Late in 1964 Reynolds and Back experimented with monomethylhydrazine, a high energy fuel to be used in the NASA Advanced Syncom and other space vehicles. The results of this toxic hazards research will be published in the near future (ref 12).

In looking to toxicological research of the future and the manner in which the psychologist can best contribute as an interdisciplinary team member, the following areas of concern will be discussed.

DESIGN CONSIDERATIONS

General

Attention should always be given to the economics of experimentation, but this is sometimes neglected. It is unfortunately the rule rather than the exception that toxicological research facilities, which are not to be singled out as unique, are usually designed without the benefit of prior planning on the part of the various disciplines to be involved, the distances that separate investigators and the numbers and kinds of personnel which may eventually be required to effectively accomplish research objectives.

Specific

The amount of space for cages, the manner in which they must be constructed, the potential interaction between subjects, the degree of visual observation possible and level of illumination are all of considerable import. It is also important to know the provisions which have been made for food and water (if not used as reinforcers of behavior), the handling of waste, electrical power (voltages, outlets and cabling), subject comfort and personnel safety. If these and other factors are considered in the early planning stages of an experimental facility, the research accomplished should be of more optimal quality.

CHOICE OF SPECIES AND SAMPLE SIZE

It would be wise for all research disciplines involved in a project to consult together before selecting a given animal for experimentation. While one species may be quite sufficient for the assessment of toxicological manifestations, it may be completely unsatisfactory for behavioral studies. In selecting animals for experimentation it would seem wise to listen to the counsel of Irwin and others who state that "interspecies differences in response. . . are least likely to occur in closely related phylogenetic forms, although there are many exceptions to this rule. Generally, however, the probability of carry-over of effects from animals to man seems to be greatest when diverse species show similar responses to a given drug, and least when their responses vary widely. It is important therefore . . . to accomplish evaluations in several species, particularly in species from different orders" (ref 3).

While a discussion of neuroanatomy and the observable correlates of behavior might be profitable for an interdisciplinary group, neither this meeting place nor the time affords such an opportunity. Let it suffice to say that from the behavioral point of view every effort should be made to utilize the information gained in preceding toxicological-physiological studies

on selected animals in determining the nervous system sites most likely to be affected by a given compound. Once such a determination is made, the psychologist should then devise performance tasks which require functions on the part of the animal that stand to elucidate the degree of nervous system impairment. Such a systematic approach is more likely to render reliable and extrapolatable data than a situation in which there is a lack of communication among the various investigators concerned.

If the psychologist is to make the best contribution possible, then the toxicologist-physiologist must consider the patterns of behavior which the species chosen is capable of emitting. Generally, most patterns of observable behavior can be divided into two types: locomotor and manipulative. In the first instance, the animal moves about in its environment; in the second instance, the animal's position is stationary but it moves objects in its environment. At the lower levels of the phyletic scale organisms have no arms or legs and thus no manipulative abilities, but they do possess locomotive abilities. In the four-limbed animals locomotion may be highly developed but in general remains similar throughout vertebrate evolution. It is late in the process of evolution that the most important changes in behavioral patterns occur -- that is, the development of manipulative ability, and this in the class mammalian. Manipulative behavior may be conveniently divided into that involving the hands, the eyes or the mouth. The excellent use of the hands, the ability to make visual discriminations without moving the head, and the coordination of the lips, tongue and vocal cords by primates, in particular, are well known (ref 8). And the nervous system changes that are involved in these evolutionary processes must be recognized -- for such recognition is vital to the proper evaluation of behavioral changes occurring in connection with toxic agents. In mammals there is an increasing dependence on the cerebral cortex for locomotor functioning. While a decorticate cat, dog, or even a monkey, can stand and walk, chimpanzees and men cannot -- but the cerebral cortex is even more essentially involved in manipulative behavior. The appearance of manipulatory behavior in evolution coincides with cortical development since it is not, as locomotive behavior is, first organized at a subcortical level and then transferred to the cortex. Since the ultimate concern of most toxicological research is man himself, it becomes increasingly clear that the best means of extrapolating to man lies in the use of those animals with advanced cortical development. Behavioral measures (performance tasks) are then really asking questions of the animal which might also be asked of man were the risk not so high. To accept anything less than this suggested paradigm may be economical, convenient and statistically sound, but it will not provide the soundest inferences of which we are capable.

In deciding upon a sample size, the power that is achieved by an N as large as six (three pairs) makes it a minimal cut-off point for behavioral studies. Tests involving subject matching and cross-over designs are thus possible, if one wishes, as well as the much used technique of the animal acting as its own control. Always, of course, a large N is desirable if it can be achieved.

SELECTION OF PERFORMANCE TASKS AND TRAINING OF SUBJECTS

The topics treated in the preceding pages have paved the way for this section. The justification for inclusion of behavioral measures and for particular kinds of performance has hopefully been achieved.

Once the psychologist has apprised himself of the research which has preceded his own on a particular compound, he sets about developing a performance schedule which should meet the following criteria:

1. The stimuli presented to the subject involve the primary sensory modalities, i. e., vision and hearing.
2. The responses required of the subject should involve those nervous system areas that have been identified in toxicological-physiological studies as potentially critical.
3. The performance schedule should not be so complex in its design that the training problems are prohibitive and become economically unfeasible to develop. Neither should the schedule be so taxing as to produce undue fatigue in single or cumulative sessions.
4. Unconditioned stimuli involving either positive or negative reinforcement should always be under experimenter control to insure that the most important question of all is answered: what is the animal capable of doing? (not: what is it simply motivated to do) -- for this is the question which confronts man in those toxic environments which pose a risk to his survival.

Training very often is approached from the point of view that asymptotic behavior is required before subjecting an animal to the experimental situation. This should only be partially true. Mogenson (ref 7) and Singh (ref 13) have both demonstrated that more highly trained responses are less susceptible to the deleterious effects of depressant drugs and that there is increasing susceptibility at lower habit strength levels (up to a point where habit no longer is greater than chance). Thus, it would appear wise to train subjects to various levels of efficiency to more accurately evaluate the effect of a given toxic agent.

The following performance schedule is an example of one recently developed for a 30-day test in a 100 per cent O₂, 5 psi environment. The stimulus-response panel for this schedule is shown in figure 1.

1. Continuous Avoidance (CA), Simple Reaction Time (RT), Auditory Discrimination (AD) and Choice Reaction Time (CRT) for 10 minutes
 - a. CA - Response-Shock Interval 5"
 - b. RT - Allowable interval 1" (4 presentations at 2, 4, 6 and 8 minutes)
 - c. AD - Allowable interval 3" (9 presentations: 8192 cps at 15" / 512 cps at 1' / 2048 cps at 2-1/2' / 512 cps at 3-1/2' / 8192 cps at 5' / 2048 cps at 6' 45" / 8192 cps at 8-1/2' / 512 cps at 9' / 2048 cps at 9' 45" /
 - d. CRT (on middle 3 IDD's and the sample) - Allowable interval 3" (9 presentations: blue at 30" / green at 1-1/2' / red at 3' / green at 4-1/2' / blue at 6' 15" / red at 7' / blue at 7-1/2' / red at 8' 45" / green at 9' 30" /
2. Three Minute Rest Period
3. Temporal Delay Task (Positive-Negative Reinforcement)

15" in which the response is withheld, 5" safety interval if a triangle is presented. 30" in which the response is withheld, 10" safety interval if a circle is presented.

Chained to Delayed Match-to-Sample Task
4. Delayed (5 seconds or more) Match-to-Sample Task

Color and form available on six Inline-Digital-Displays (IDD). A corrective procedure will be used in which only the last discrimination will be positively rewarded. Use red, blue, orange, yellow, purple and brown - and parallel bars, verticle line, horizontal line, plus, a number sign, and an equal sign for a total of 24 problems. The sample will remain on for 5" before being extinguished.

Chained to Discrimination Reversal Task
5. Discrimination Reversal Task

On the upper left two round pushbutton IDD units there will be 15 problems of a non-corrective nature

(Series I). Each correct choice will be positively rewarded with a food pellet. Upon completion of the first series of problems the task will switch to the two upper right pushbutton IDD's (Series II) for a reversal of the problem, i.e., the previous incorrect response will become the correct response and the animal must reverse its behavioral pattern to obtain a reward. The programming unit should permit the alternation of these series if the experimenter wishes.

6. The amount of rest after 3. - 5. is dependent upon the animal's progress, but work plus rest will not exceed one hour. It is anticipated that the work period will ordinarily be about 30 minutes in duration.

While this performance schedule was developed for a chimpanzee, a Macaque monkey could learn these tasks in a somewhat longer period of time. If one wishes to consider the minimum (especially with a larger N) performance measures to facilitate testing, then it is recommended that a continuous motor task, a visual reaction time measure, and a measure of auditory reaction time comprise the schedule. For a slightly more sophisticated schedule one could include choice reaction time (visual and/or auditory) and an oddity problem. And for something just short of the complex schedule outlined previously, a timing problem or learning set series could be incorporated.

Figures 2 and 3 show the 6571st Aeromedical Research Laboratory's current toxic hazards experimental room and a psychophysical programming apparatus to be used in future toxicological-pharmacological research.

PROGRAMMING TASKS AND DATA ACQUISITION

The experience gained in some four years of research with space-related variables suggests that conventional relay circuitry provides the flexibility needed during animal training, but that solid state circuitry is necessary for accuracy of timing functions, environmental testing extremes, climatic variation, long-duration usage and overall reliability. There are two or three reputable companies where such electronic equipment is available. Data are normally accumulated on print-out counters, running time meters, digital counters and cumulative response recorders.

DATA ANALYSIS

All too often there is undue emphasis given to group as opposed to individual subject responses. This may be an unwise approach since eventually

one must deal with individuals and not groups, and group data have little meaning for individual therapy. In studies on locomotor and conditioned-avoidance behavior, Irwin (ref 2) and Irwin and Slabok (ref 4) have been able, through various statistical techniques, to minimize certain potentially relevant variables and to increase the precision of their analyses.

INTERPRETING FINDINGS

Whether it is justified or not, it is usual to extrapolate the findings from animal research to man, since it is assumed that a number of the behaviors found in man are also present in higher animals. This is true, at least, in much of psychopharmacological research and thus has become the working hypothesis of most investigators (ref 3). But may it be said that such a working hypothesis will prove scientifically sound only to the extent that the behavior patterns examined in animals are representative of those found in man. Animal responses measured without regard to evolutionary nervous system changes manifested in, e.g., manipulatory behavior, can only result in quantitative and qualitative variations from man's responses. In the study of toxic environments which pose a high risk to human life, it is essential that this pitfall be avoided.

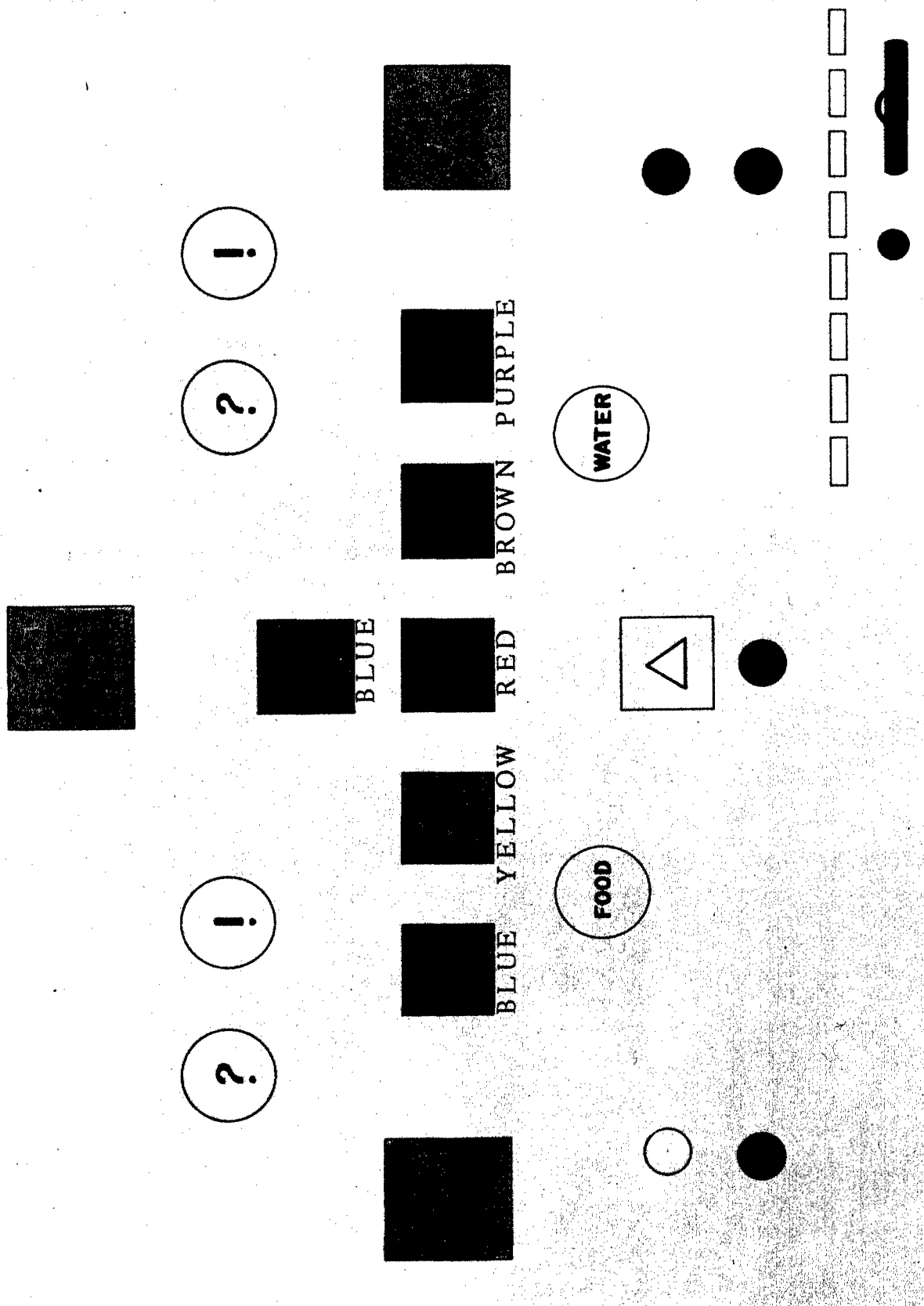


FIGURE 1. REPRESENTATIVE ANIMAL PERFORMANCE PANEL FOR COMPLEX SCHEDULE



FIGURE 2. TOXIC HAZARDS EXPERIMENTAL ROOM

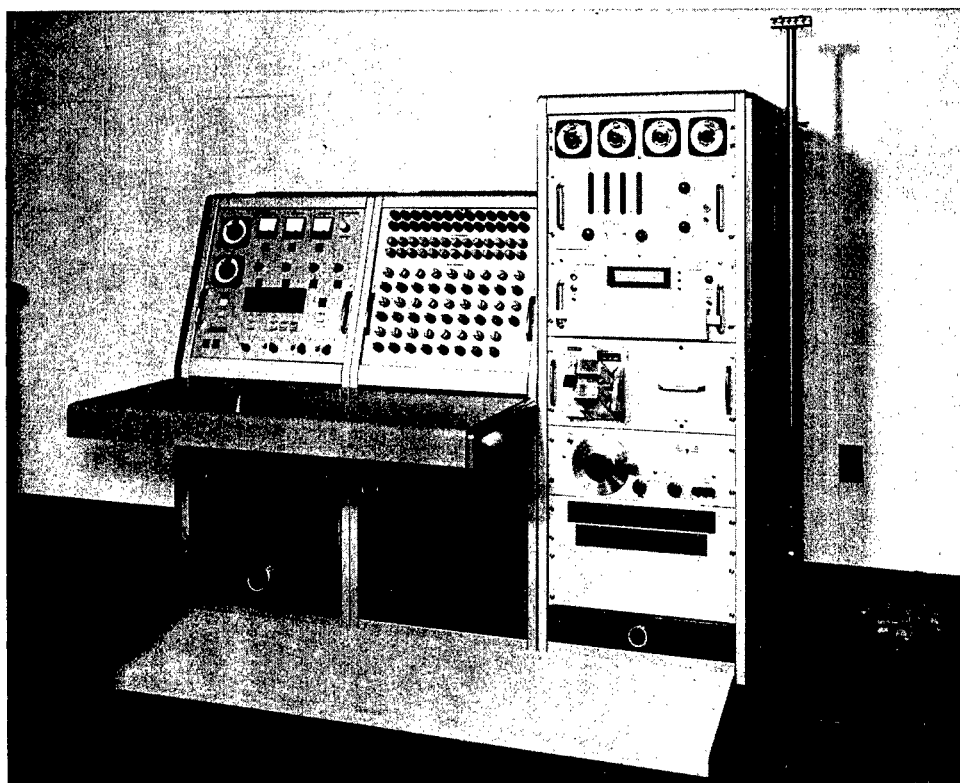


FIGURE 3. PSYCHOPHYSICAL APPARATUS FOR TOXICOLOGICAL-PHARMACOLOGICAL RESEARCH

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PRELIMINARY RESULTS OF TOXICITY STUDIES IN 5 PSIA 100% OXYGEN ENVIRONMENT

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Introduction

A concentrated research effort was made during the first six months of operation of the Altitude Facility of the Toxic Hazard Research Laboratory to answer two fundamental questions:

1. Is a 90-day continuous exposure to an environment of 100% oxygen at a reduced pressure of 5 psia (pounds per square inch, absolute) harmful based upon its effects on laboratory animals?
2. Are contaminants under conditions of 5 psia pressure and 100% oxygen more toxic than they are in air under normal atmospheric pressures? (Normal atmospheric pressures which are variable in nature were held constant in the inhalation exposure chambers at 13.5 psia. The pressure was controlled at slightly less than that of the ambient conditions so that any leak in the exposure apparatus would be in board.)

Materials and Methods

A series of continuous exposure studies was designed in an attempt to answer the above questions. A list of studies which have been completed in this program are listed in Table 1.

The particular contaminants were chosen primarily for the following reasons:

1. Carbon Tetrachloride: To determine if a systemic toxicant that has no apparent pulmonary irritating effects at the concentrations employed exhibits more pronounced enzymatic and histologic effects at 100% oxygen and 5 psia, hereafter referred to as altitude conditions, than at 13.5 psia and air, hereafter referred to as ambient conditions.
2. Nitrogen Dioxide: To determine if a pulmonary irritant will produce a more pronounced irritating effect at altitude conditions than at ambient conditions.

3. Ozone: To determine if a pulmonary irritant that also exhibits systemic effects proves to be more toxic at altitude conditions than at ambient conditions.

In Table 2 is presented the routine animal loading of the four domes (the inhalation exposure chambers designed specifically for exposures to various air-oxygen mixtures at reduced pressures). Domes No. 1, 2 and 3 were employed to expose animals to the contaminants under either altitude or ambient conditions while Dome No. 4 was used to expose both the 90-day Oxygen Toxicity Study animals and the 14-day Oxygen Toxicity Study animals, the latter being introduced and removed at the beginning and end of each 14-day contaminant study. The 14-day Oxygen Toxicity Study animals, together with animals maintained in air-conditioned animal rooms, served as controls for the 14-day contaminant studies. Since Dome 4 served the dual purpose of simultaneously holding the animals from two studies, it was necessary to increase its animal load; however, neither the carbon dioxide concentration nor the temperature in Dome 4 was significantly different from that in the other three domes.

Records were maintained on mortality, growth, hematology, clinical chemistry, organ weights and histopathology. All but histopathology is reviewed in this presentation.

In maintaining records, the following protocol was employed. Body weights were determined on rats, beagles and monkeys during their acclimatization period, on the first day of their exposure and on the final day of their exposure, at which time they were sacrificed. Blood was drawn from the beagles and monkeys for hematological and biochemical evaluation. Each of these animals had a minimum of three baselines determined at weekly intervals prior to exposure so as to insure the use of healthy, normal animals. Another hematological and biochemical baseline was obtained immediately before the beginning of the exposure and a final one immediately following completion of the exposure. Table 3 lists the tests performed by our Medical Technology Department. After initiation of the exposure, each of the animals was observed at thirty-minute intervals, twenty-four hours per day for signs of irritation and the occurrence of death. Upon autopsy of the animals which died during exposure or were sacrificed at its termination, liver, kidney, spleen, heart, lung, and in some cases, the brain, were removed, weighed, and sections of these and other tissues judged pertinent to the study were removed for histopathological study.

Results - 90-day Oxygen Toxicity Study

In Table 4 is presented the mortality data collected during the 90 days of continuous exposure in a 5 psia and 100% oxygen environment. The reduction in the number of animals exposed with each time period is due to a number of animals of each species being sacrificed after approximately 14, 30 and 60

days for the purpose of serial study. The exposure had no lethal effect on beagles and monkeys, a minimal effect on mice and a marked effect on rats. The death of the rats was strain-specific which was proven in a subsequent corollary study, the results of which are presented in Table 5. No mortality occurred during seven days of continuous exposure of the Sprague-Dawley-derived rats (Carworth Farms, CFE, specific pathogen free), while Wistar-derived rats (procured from the same supplier and of the same size as those employed in the 90-day study) exhibited approximately the same percentage mortality as produced in the 90-day study. The significantly lower mortality produced in the case of the old male Wistars points to the possibility that the Wistar rats may overcome this particular sensitivity with age.

No adverse effect on growth or organ weights was noted during or following exposure.

In Table 6, the effect of the exposure upon the serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) levels of beagles and monkeys is present. Although most of the transaminase means are within two standard deviations of the mean values of the control animals, there is a notable increase exhibited in the case of beagles. The control data parameters presented are based upon a statistical analysis of all of the pre-exposure data collected on all of the experimental and control animals studied during our first 16 dome runs. The trend shown in Table 6 for the means of groups of beagles is even more strikingly shown in Table 7 where the data for individual dogs are presented and exemplify the use of an animal as its own control.

Contaminant Studies in a 5 P. S. I. A. - 100% Oxygen Environment

The effect of each of the contaminants plus its environment on laboratory animals will be treated consecutively.

1. Carbon Tetrachloride

In Table 8 is presented the mortality that occurred during exposures of animals to several different concentrations of the contaminant. Significant mortality was produced in mice at 594 mg/m^3 concentration level. The rat mortality was (as is noted in the table, and explained previously) due to the apparently altitude-sensitive Wistar-derived strain. The relatively uniform mortality throughout the three concentration-level groups and the altitude control group confirms that the mortality effect was not caused by the contaminant.

A correlation was exhibited between increased contaminant concentration and decreased growth as is shown in Table 9.

The organ weights were found to be not significantly different from those of control animals.

The effects of the exposures of carbon tetrachloride at altitude conditions on SGPT, SGOT and Alkaline Phosphatase levels in male beagles, female beagles and monkeys are found in Tables 10, 11 and 12, respectively. These were the only clinical chemistry parameters studied which were significantly affected. In Table 10, the male beagles show a slight increase in SGPT levels with increase in contaminant concentration even in the lower concentration levels. While at the 594 mg/m³ concentration level, SGPT, SGOT and Alkaline Phosphatase levels have been significantly increased over these of control animals. The female beagles exhibited the same increasing trend in SGPT levels and significant changes in SGPT, SGOT and Alkaline Phosphatase at 594 mg/m³ concentration level as is shown in Table 11. In the case of monkeys, however, only the SGPT level showed a significant change and that only at the 594 mg/m³ concentration level. The SGOT and Alkaline Phosphatase levels were essentially unaffected.

2. Nitrogen Dioxide

In Table 13 is presented the mortality produced during exposures to several concentration levels of nitrogen dioxide. It should be noted that the monkey proved to be more sensitive to NO₂ than the beagle. It was observed during the early stages of the 38 mg/m³ concentration level exposure that both the beagles and monkeys were severely affected. The beagles rapidly recovered after one to two days to an almost normal condition, whereas the two surviving monkeys showed signs of severe strain throughout the entire exposure period.

An association was exhibited between increased contaminant concentration and decreased growth as is shown in Table 9.

No notable effect was found by an examination of either the clinical chemistry, hematological or the organ weight data.

3. Ozone

The mortality produced in animals during exposures to several concentration levels of ozone is presented in Table 14. Guinea pigs were also exposed in the ozone studies since they have been reported to be particularly sensitive to this chemical. It should be noted that in contradistinction to the effects of nitrogen dioxide, the beagles were more highly affected than the monkeys. Why nitrogen dioxide is more lethal to monkeys than to beagles and why ozone is more lethal to beagles than to monkeys is a question worthy of further investigation. During the first day or two of exposure to ozone, the monkeys appeared to be under severe stress but rapidly recovered.

An association was exhibited between increased contaminant concentration and decreased growth as is shown in Table 9. No significant changes were found in the clinical chemistry or hematological data. The post exposure

lung weights were significantly affected by increasing ozone concentration as is shown in Table 15. This was expected, due to the pulmonary edemogenic nature of the gas.

Contaminant Studies, Altitude Versus Ambient Conditions

The final series of results is a comparison of the effects on animals of altitude versus ambient conditions employing approximately the same concentration of contaminants. Two levels were chosen, one which will be referred to as "High Level" concentrations and the other as "Low Level" concentrations.

A comparison of the mortality effects produced by "High Level" concentrations of the three contaminants under altitude versus ambient conditions is presented in Table 16.

The carbon tetrachloride data presents two points worthy of note: (1) the chemical is apparently more toxic to mice under altitude conditions, whereas in every other case the contaminants under altitude conditions are no more toxic or less toxic than under ambient conditions, and (2) the absence of mortality in the group of exposed rats. These rats were of the Sprague-Dawley-derived, specific pathogen free strain, rather than the Wistar-derived strain that had previously been shown to be sensitive to altitude conditions.

Both nitrogen dioxide and ozone, under altitude conditions, appear to be less toxic than under ambient conditions. This difference in toxic effect is further shown by an examination of the times of the occurrences of deaths as is presented in Tables 17 and 18. Under altitude conditions, the animals survived for longer periods of exposure.

A comparison of the mortality produced by the "Low Level" concentrations of the three contaminants under altitude versus ambient conditions is presented in Table 19.

The eight rats which died in the carbon tetrachloride altitude study were Wistar-derived whereas those referred to in the ambient study were Sprague-Dawley-derived.

Nitrogen dioxide and ozone appeared to be less toxic under altitude conditions than under ambient conditions (especially in the case of the beagles and monkeys). These data present very clearly the difference in species response, i. e., the monkeys being more sensitive than beagles to the effects of nitrogen dioxide and beagles being more sensitive than monkeys to the effects of ozone.

None of the "Low Level" concentrations of the three contaminants produced effects on the hematological or the clinical chemistry profiles of the

surviving exposed animals that were significantly different from those of control animals. The "High Level" concentrations of carbon tetrachloride and ozone, however, did produce significant changes; the former affecting the SGPT, SGOT and Alkaline Phosphatase levels in beagles as presented in Table 20, whereas the latter depressed the Alkaline Phosphatase levels in the surviving monkeys (all of the beagles having died during the exposure). The mean Alkaline Phosphatase level for the surviving monkeys which were exposed to 14.9 mg/m^3 of ozone at ambient conditions was 18 units with a range of 14 to 23 units, whereas their controls maintained in air-conditioned animal quarters exhibited a mean of 34 units with a range of 22 to 42 units. The monkeys exposed to 15.4 mg/m^3 of ozone at altitude conditions had a mean Alkaline Phosphatase level of 14 units with a range of 8 to 23 units, whereas their two controls exposed to 100% oxygen at altitude conditions both exhibited a mean of 25 units.

No clear-cut difference between the effect of altitude and ambient conditions on the toxicity of carbon tetrachloride is evident in Table 20.

Summary and Conclusions

A 90-day continuous exposure of mice, rats, beagles and monkeys to a 5 psia and 100% oxygen environment produced the following pertinent results:

1. A Wistar-derived strain of rats proved to be sensitive to altitude conditions early in the exposure (15% mortality within 14 days of exposure) whereas a Sprague-Dawley-derived strain proved resistant.
2. A possible association of increasing SGPT levels in beagles with length of exposure was found.

Except for these factors, the experimental animals gave no apparent indication of being stressed throughout the exposure. A one-year study has been initiated to determine if the enzyme change was due to sampling or is indicative of an accumulating stress.

No significant increase in the toxic response of animals to inhaled atmospheric contaminants under conditions of 5 psia and 100% oxygen was noted when compared with animals exposed under normal atmospheric conditions (except in the case of mice exposed to carbon tetrachloride).

Based upon mortality data, a definite reduction in toxic response to pulmonary irritants was found in the presence of reduced pressure (5 psia) and 100% oxygen when compared with ambient pressure at the same concentration for two weeks continuous exposure. This difference in toxic response may be a beneficial effect derived from the increased partial pressure of oxygen in the experimental chambers even though total pressure has been reduced.

Specifically, this is an increase in oxygen partial pressure from approximately 150 millimeters Hg pO₂ to 255 millimeters Hg pO₂. This increase in oxygen tension at the pulmonary surface may be acting therapeutically against the pulmonary edema produced by the lung irritants, ozone and nitrogen dioxide. In the case of carbon tetrachloride, a systemic toxicant, no such benefits were observable.

TABLE 1
CONTINUOUS EXPOSURE STUDIES

- I. 90-day Oxygen Toxicity Study (5 psia, 100% O₂)
- II. 14-day Oxygen Toxicity Studies (5 psia, 100% O₂)
- III. 14-day Contaminant Studies (5 psia, 100% O₂)

CCl ₄	NO ₂	O ₃
1. 13 mg/m ³	1. 10 mg/m ³	1. 1.9 mg/m ³
2. 32 mg/m ³	2. 17 mg/m ³	2. 4.2 mg/m ³
3. 80 mg/m ³	3. 38 mg/m ³	3. 8.0 mg/m ³
4. 594 mg/m ³	4. 81 mg/m ³	4. 15.4 mg/m ³

- IV. 14-day Contaminant Studies (13.5 psia, air)

CCl ₄	NO ₂	O ₃
1. 35 mg/m ³	1. 36 mg/m ³	1. 7.9 mg/m ³
2. 577 mg/m ³	2. 89 mg/m ³	2. 14.9 mg/m ³

TABLE 2
ROUTINE ANIMAL LOADING OF DOMES

Dome No.	Species	No. of Cages	Animals/Cage	Total
1, 2 + 3	Monkeys	4	1	4
	Beagles	2	4	8 (4M + 4F)
	Rats	8	6 or 7	50 (25M + 25F)
	Mice	2	20	40
	Guinea Pigs*	2	4	8
4	Monkeys	8	1 or 2	10
	Beagles	2	5	10 (5M + 5F)
	Rats	10	10	100 (50M + 50F)
	Mice	5	20	100
	Guinea Pigs*	2	4	8

M = males

F = females

* Guinea Pigs were employed only in the Ozone Studies.

TABLE 3

CLINICAL LABORATORY TESTS ROUTINELY PERFORMED

I. Hematology

1. Total WBC (cells/mm³)
2. Differential (per 100 cells)
3. Total RBC (million cells/mm³)
4. Hemoglobin (gm %)
5. Hematocrit (vol %)

II. Clinical Chemistry

1. Sodium (meq/l)
2. Potassium (meq/l)
3. Calcium (meq/l)
4. Total Protein (gms %)
5. Albumin (gm %)
6. SGPT (Reitman-Frankel units)
7. SGOT (Reitman-Frankel units)
8. Alkaline Phosphatase (Reid, Klein, Babson units)
9. Total Phosphorus (mg %)
10. Total LDH (Wroblewski units/ml serum)

TABLE 4

MORTALITY DURING 90-DAY CONTINUOUS EXPOSURE IN 5 P. S. I. A.
100% OXYGEN ENVIRONMENT

Days of Continuous Exposure	Number Dead/Number Exposed			
	Mice	Rats*	Beagles	Monkeys
14	1/100	15/100	0/10	0/10
28	0/59	1/53	0/8	0/8
60	2/49	5/44	0/6	0/6
91	3/40	1/32	0/4	0/4
Total Deaths	6	22	0	0

* Wistar-derived strain

TABLE 5

MORTALITY EFFECT OF ALTITUDE (100% O₂, 5 PSIA) CONDITIONS
ON WISTAR AND SPRAGUE-DAWLEY DERIVED RATS

Day of Continuous Exposure	Occurrence of Deaths by Rat Species			
	Male (20)	Wistar-Derived Female (20)	Old Male (20)	Sprague-Dawley-Derived Male (40)
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	5	3	1	0
5	1	0	0	0
6	0	1	0	0
7	0	0	0	0
TOTALS	6/20	4/20	1/20	0/40

TABLE 6

EFFECT OF 90-DAY CONTINUOUS EXPOSURE IN 5 P.S.I. A. - 100% OXYGEN
ENVIRONMENT ON SGPT AND SGOT VALUES

Days of Exposure	Mean SGPT Values		Mean SGOT Values	
	Beagles	Monkeys	Beagles	Monkeys
14	24	35	22	43
28	21	29	24	35
60	30	35	29	43
91	33	29	28	40

Control Data (Mean \pm 2 Std. Dev.):

	SGPT	SGOT
Beagles	17 \pm 15	24 \pm 22
Monkeys	27 \pm 13	36 \pm 20

TABLE 7
SGPT TRENDS IN INDIVIDUAL BEAGLES

Beagle No.	Baseline (Air) 15 P. S. I.					Days in 5 P. S. I. Oxygen		
	1	2	3	4	Avg.	30	60	90
P-3	20	16	11	20	17	22		
ZAN-2	16	16	25	16	18	20		
RN-1	22	22	11	26	15		31	
V-532	20	16	25	22	21		28	
A-26	--	--	22	--	22		31	
A-30	--	--	32	--	32		31	
6-242	9	9	9	--	9		18	
OL-2	9	--	11	--	10		31	
M-1	20	11	16	16	16			31
M-3	16	22	16	22	19			35
350-6	11	20	22	11	16			28
362-2	20	16	22	26	21			39

TABLE 8

MORTALITY PRODUCED BY A 14-DAY CONTINUOUS EXPOSURE TO
CARBON TETRACHLORIDE, 5 PSIA, 100% OXYGEN ENVIRONMENTS

Conc. (mg/m ³)	Number Dead/Number Exposed			
	Mice	Rats	Beagles	Monkeys
<u>Controls</u>				
0 (Ambient)	0/40	*0/40	---	---
0 (Altitude)	0/40	*8/40	0/2	0/2
<u>Contaminant</u>				
13	0/40	*6/50	0/8	0/4
32	0/40	*8/50	0/8	0/4
80	0/40	*7/50	0/8	0/4
594	39/40	+0/50	0/8	0/4

* Wistar-derived, shown to be species-sensitive to altitude conditions in corollary study.

+ Sprague-Dawley-derived rats employed in this and subsequent studies.

TABLE 9

RAT BODY WEIGHT CHANGES DURING CCl_4 , NO_2 AND O_3 STUDIES
(14-DAY CONTINUOUS EXPOSURE, 100% O_2 , 5 P.S.I.A.)

CCl_4			NO_2			O_3		
Conc. mg/m^3	Wgt. Change (gms/day) M	F	Conc. mg/m^3	Wgt. Change (gms/day) M	F	Conc. mg/m^3	Wgt. Change (gms/day) M	F
<u>Controls</u>								
0. (Ambient)	5.5	2.5	0. (Ambient)	2.1	2.2	0. (Ambient)	4.3	2.3
0. (Altitude)	3.6	1.4	0. (Altitude)	4.4	2.4	0. (Altitude)	0.5	0.8
<u>Contaminant</u>								
13	3.8	2.6	10	5.7	2.7	1.9	2.9	1.3
32	3.6	1.6	17	5.2	2.6	4.2	1.1	0.6
80	3.2	1.5	38	4.4	1.9	8.0	---	3.0
594	-0.9	-1.3	81	-1.9	-1.6	15.4	---	---

TABLE 10

EFFECT OF 14-DAY CONTINUOUS CCl_4 EXPOSURES IN 5 P.S.I.A. 100% OXYGEN
ENVIRONMENT ON SGPT, SGOT AND ALKALINE PHOSPHATASE LEVELS
IN MALE BEAGLES

Conc. ³ mg/m ³	P. S. I. A.	Environment	SGPT (Mean + Range)	SGOT (Mean + Range)	Alk. Phos. (Mean + Range)
0	5	100% O ₂	24 (----)	24 (----)	---
13	5	100% O ₂	26 (20-32)	27 (24-32)	---
32	5	100% O ₂	27 (11-46)	30 (14-42)	---
80	5	100% O ₂	31 (20-41)	29 (24-37)	---
594	5	100% O ₂	324 (250-465)	115 (95-150)	24 (14-34)

Control Data (Mean \pm 2 Std. Dev.):

SGPT: 15 ± 16 SGOT: 23 ± 14 Alk. Phos.: 3 ± 3

TABLE 11

EFFECT OF 14-DAY CONTINUOUS CCl_4 EXPOSURES IN 5 P. S. I. A. 100% OXYGEN
ENVIRONMENT ON SGPT, SGOT AND ALKALINE PHOSPHATASE LEVELS
IN FEMALE BEAGLES

Conc. ³ mg/m ³	P. S. I. A.	Environment	SGPT (Mean + Range)	SGOT (Mean + Range)	Alk. Phos. (Mean + Range)
0	5	100% O ₂	24 (----)	20 (----)	---
13	5	100% O ₂	21 (20-22)	29 (24-37)	---
32	5	100% O ₂	24 (20-32)	29 (20-32)	---
80	5	100% O ₂	29 (26-32)	36 (28-49)	---
594	5	100% O ₂	503 (290-680)	127 (108-170)	21 (10-27)

Control Data (Mean \pm 2 Std. Dev.):

SGPT: 19 ± 13 SGOT: 25 ± 30 Alk. Phos.: 3 ± 4

TABLE 12

EFFECT OF 14-DAY CONTINUOUS CCl_4 EXPOSURES IN 5 P.S.I.A. 100% OXYGEN ENVIRONMENT ON SGPT, SGOT AND ALKALINE PHOSPHATASE LEVELS IN MONKEYS

Conc. mg/m ³	P. S. I. A.	Environment	SGPT (Mean + Range)	SGOT (Mean + Range)	Alk. Phos. (Mean + Range)
0	5	100% O ₂	35 (28-42)	43 (37-49)	---
13	5	100% O ₂	45 (37-56)	69 (55-86)	---
32	5	100% O ₂	38 (22-83)	59 (32-120)	---
80	5	100% O ₂	37 (32-41)	56 (37-70)	---
594	5	100% O ₂	127 (39-250)	49 (40-64)	26 (20-34)

Control Data (Mean \pm 2 Std. Dev.):

SGPT: 27 \pm 13 SGOT: 36 \pm 20 Alk. Phos.: 28 \pm 15

TABLE 13

MORTALITY PRODUCED BY A 14-DAY CONTINUOUS EXPOSURE TO
NITROGEN DIOXIDE, 5 PSIA, 100% OXYGEN ENVIRONMENTS

Conc. (mg/m ³)	Number Dead/Number Exposed			
	Mice	Rats	Beagles	Monkeys
<u>Controls</u>				
0 (Ambient)	0/40	0/40	---	---
0 (Altitude)	0/40	0/40	0/4	0/4
<u>Contaminant</u>				
10	5/40	0/50	0/8	0/4
17	0/40	0/50	0/8	0/4
38	0/40	3/50	0/8	2/4
81	40/40	37/50	7/8	4/4

TABLE 14

MORTALITY PRODUCED BY A 14-DAY CONTINUOUS EXPOSURE TO
OZONE , 5 PSIA, 100% OXYGEN ENVIRONMENTS

Conc. (mg/m ³)	Mice	Number Dead/Number Rats	Exposed G. Pigs	Beagles	Monkeys
<u>Controls</u>					
0 (Ambient)	0/40	0/50	0/8	---	---
0 (Altitude)	3/20	3/20	0/8	0/4	0/4
<u>Contaminant</u>					
1.9	0/40	0/50	0/8	0/8	0/4
4.2	0/40	4/50	0/8	1/8	0/4
8.0	33/40	45/50	8/8	2/8	0/4
15.4	---	---	---	6/8	0/4

TABLE 15

EFFECT OF OZONE ON LUNG WEIGHTS
(14-DAY CONTINUOUS EXPOSURE, 100% O₂, 5 PSIA)

Conc. (mg/m ³)	Lung Weight (wet)/Body Weight Ratios				
	Rat (mg/gm)		Beagle (gm/kg)		Monkey (gm/kg)
	Male	Female	Male	Female	Female
<u>Controls</u>					
0 (Ambient)	6	6	--	--	--
0 (Altitude)	29	10	16	10	6
<u>Contaminant</u>					
1.9	8	9	10	12	8
4.2	12	10	15	12	7
8.0	21	24	29	20	10
15.4	--	--	34	--	14

TABLE 16

MORTALITY PRODUCED BY "HIGH-LEVEL" CONCENTRATIONS OF CCl_4 , NO_2 AND O_3 ALTITUDE
(100% O_2 , 5 PSIA) VS. AMBIENT (AIR, 13.5 PSIA) CONDITIONS

	Conditions	Conc. (mg/m^3)	Mice	Number Dead/Number Exposed		
				Rats	G. Pigs	Beagles
CCl_4	Alt.	594	39/40	0/50	---	0/8
	Amb.	577	2/40	0/50	---	0/8
NO_2	Alt.	81	40/40	37/50	---	7/8
	Amb.	89	40/40	50/50	---	8/8
O_3	Alt.	15.4	-----*	-----*	-----*	6/8
	Amb.	14.9	40/40	50/50	8/8	8/8
						0/4
						1/4

* These species were not exposed at this concentration because of expected 100% mortality.

TABLE 17

MORTALITY PRODUCED BY NITROGEN DIOXIDE
ALTITUDE (100% O₂, 5 PSIA) VS. AMBIENT (AIR 13.5 PSIA) CONDITIONS

Day of Continuous Exposure	Conc. (mg/m ³)		Occurrence of Deaths by Species							
	Alt.	Amb.	Mice (40)		Rats (50)		Beagles (8)		Monkeys (4)	
			Alt.	Amb.	Alt.	Amb.	Alt.	Amb.	Alt.	Amb.
1	83	83	0	0	0	41	0	7	4	4
2	74	87	9	3	8	9	2	1		
3	80	84	8	21	7		2			
4	89	94	2	16	6		0			
5	88	95	1		1		2			
6	76		6		1		0			
7	85		4		2		0			
8	83		1		2		1			
9	98		2		4		0			
10	86		2		1		0			
11	56		3		1		0			
12	81		1		1		0			
13	80		0		1		0			
14	75		1		2		0			
TOTALS	(81)	(89)	40	40	37	50	7	8	4	4

TABLE 18

MORTALITY PRODUCED BY OZONE
ALTITUDE (100% O₂, 5 PSIA) VS. AMBIENT (AIR, 13.5 PSIA) CONDITIONS

Day of Continuous Exposure	Conc. (mg/m ³)		Occurrence of Deaths by Species			
	Alt.	Amb.	Beagles (8)		Monkeys (4)	
			Alt.	Amb.	Alt.	Amb.
1	17.7	15.5	0	3	0	1
2	14.7	15.2	2	4	0	0
3	14.8	15.1	0	0	0	0
4	15.7	15.4	2	0	0	0
5	15.3	16.4	0	0	0	0
6	13.5	12.7	0	0	0	0
7	15.7	11.6	2	1	0	0
8	15.7	16.7	0		0	0
9	15.5	15.0	0		0	0
10	14.4	14.6	0		0	0
11	13.7	15.3	0		0	0
12	16.7	16.1	0		0	0
13	15.7	14.1	0		0	0
14	15.7	14.4	0		0	0
TOTALS	(15.4)	(14.9)	6	8	0	1

TABLE 19

MORTALITY PRODUCED BY "LOW-LEVEL" CONCENTRATIONS OF CCl_4 , NO_2 AND O_3 ALTITUDE
(100% O_2 , 5 PSIA) VS. AMBIENT (AIR, 13.5 PSIA) CONDITIONS

	Conditions	Conc. (mg/m^3)	Mice	Number Dead/Number Exposed			Monkeys
				Rats	G. Pigs	Beagles	
CCl_4	Alt.	32	0/40	*8/50	---	0/8	0/4
	Amb.	35	0/40	0/50	---	0/6	0/4
NO_2	Alt.	38	0/40	3/50	---	0/8	2/4
	Amb.	36	2/40	7/50	---	0/6	4/4
O_3	Alt.	8.0	33/40	45/50	8/8	2/8	0/4
	Amb.	7.9	33/40	50/50	8/8	5/5	2/4

* Wistar-derived rats

TABLE 20

EFFECT OF 14-DAY CONTINUOUS "HIGH-LEVEL" CCl_4 EXPOSURES ON THE
SGPT, SGOT AND ALKALINE PHOSPHATASE LEVELS IN LABORATORY ANIMALS
ALTITUDE VS. AMBIENT CONDITIONS

Conditions	Conc. ³ (mg/m ³)	SGPT (Mean + Range)	SGOT (Mean + Range)	Alk. Phos. (Mean + Range)
<u>Male Beagles</u>				
Altitude	594	324 (250-465)	115 (95-150)	24 (14-34)
Ambient	577	461 (300-818)	94 (74-135)	12 (7-21)
<u>Female Beagles</u>				
Altitude	594	503 (290-680)	127 (108-170)	21 (10-27)
Ambient	577	539 (348-818)	106 (74-150)	11 (6-18)
<u>Monkeys</u>				
Altitude	594	127 (39-250)	49 (40-64)	26 (20-34)
Ambient	577	44 (30-60)	54 (45-64)	28 (26-33)

REVIEW OF AIR FORCE DATA FROM LONG TERM CONTINUOUS EXPOSURE AT AMBIENT PRESSURE

By

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Mr. Chairman, Ladies and Gentlemen, I will be reporting today on work which has been performed under Air Force sponsorship in the area of environmental toxicology of space cabin atmospheres over the past 5 years. Indeed, this early work gave us the warning that materials in trace quantities could prove toxic when presented to animals over long continuous exposure periods, and in a sense, (this early work) is the reason we are here today. Many of you may know that this work was performed at Midwest Research Institute in Kansas City, Missouri, during a period from 1 March 1960 to 1 June 1963. The first 2 years' work was performed by Dr. Curtis Sandage, and the last year's work was performed by Dr. William B. House.

Our original concern stemmed from the fact that space cabin engineers had been requesting information regarding criteria which would allow them to pick optimum materials to use in space cabins. The engineer recognized early that space cabin materials would probably produce gas-off vapors and noxious gases which would not be too different from the problems that the Navy occasionally had in their submarine program. Of course, the ideal system for solving these problems would be to remove completely all contaminants from a closed system, but practical considerations of the power necessary to accomplish this showed that in at least our earlier systems, this was not feasible. Engineers, for lack of better data, had been using highly speculative figures from Industrial Threshold Limit Values (TLV) for establishing tolerable contaminant concentration levels.

For example, values of 10 ppm were being cited for carbon monoxide, hydrogen, methane and para-cresol; 50 ppm for hydrogen sulfide, indole, skatole, ammonia and methyl mercaptan. Statements to the effect that organic pollutants should not be allowed to rise above 100 ppm, inorganics above 10 ppm, heavy metals above 1 ppm and halogenated compounds such as hydrogen fluoride and chloride above 3 ppm for periods of 24 hours or more were advanced as criteria for engineering purposes. Unfortunately, the TLV is designed to be used only for 8-hour exposures during a 5-day work week, and over a 30-year work span. Those of us oriented in toxicology immediately recognized that any extrapolation of these values to

30-, 60-, 90-or-more-day continuous exposure periods would be highly suspect, even though many of these values have large built-in safety factors. Therefore, our initial plan was to perform some valid experiments which would either prove our original assumption that TLV's were poor or unusable values for extrapolation, or that maybe we could apply some factor(s) to these values and produce some usable long term, continuous exposure criteria. To this end we decided first to study a group of metabolic products -- a mixture of indole, hydrogen sulfide, methyl mercaptan and skatole -- to see if an atmosphere containing these well-known metabolites would pose a toxicological problem. At the same time, we chose two compounds -- phenol and carbon tetrachloride -- to be run concurrently with our mixture. Carbon tetrachloride was picked because down through the years its TLV has been reduced many times as toxicological experience dictated. Phenol, on the other hand, had a good stable TLV history.

At this time then, we would like to discuss the first exposure chambers which were used for these experiments. Four exposure chambers were constructed for this work. This permitted us to use one for a control group of animals and three for contaminant exposure chambers. Each chamber was 10 feet long, 8 feet wide and 7 and 1/2 feet high. Each room was heavily insulated, the internal walls and ceiling being covered with double aluminum "sandwich-type" insulation material. The chambers were entered through a door placed in the middle of each room. As you can see, these chambers are a "far cry" from those which we are presently using. However, we made a good attempt at supplying each chamber with cleaned air by separate one-ton air-conditioning units placed on top of each isolation chamber. Thermostatic controls for each unit were placed inside the chambers, and no chamber air was recirculated. The volume of air turnover was approximately one-tenth of the room volume per minute. A fail safe control system was devised to maintain constancy of chemical input into each room. Each chamber was equipped with sampling tubes for monitoring internal atmospheres from the outside of the chambers. Experimentation proved that concentrations of contaminants were equally mixed throughout each chamber. Good mixing was insured by small fans mounted within the chambers. Monkeys, rats and mice were housed in cages arranged linearly along one wall of the room, a marked departure from our presently used cages and arrangement. Four of the 6 sides of each cage were solid. Each room contained 10 monkeys, 50 rats and 100 mice. All animals were followed by a number of clinical laboratory examinations before, during and following the 90-day exposure, and the animals were terminally given stress tests which I will discuss a little later, and then necropsied with both gross and microscopic examination of tissues. At the end of the first 3 runs with CCl_4 (25 ppm), phenol (5 ppm) and the mixture of metabolic products, it became apparent that we would have to rerun the experiment utilizing individual components of the mixture, since we did observe pathology and death in 8 of 10 monkeys and in a number of mice. None of the necropsy findings or clinical chemistries revealed a single common cause of death in the monkey population, and the 2 monkeys that survived the

test period were in remarkably good condition. Therefore, we repeated the test and exposed a like group of animals again to a mixture of the metabolites and, this time, one group to hydrogen sulfide (20 ppm), one to methyl mercaptan (50 ppm) and one to indole (10.5 ppm). A control group received no agent. Skatole was not given separately because we did not have chamber space, and skatole is chemically only one methyl group from indole and, we thought, probably not too different toxicologically. I would like to present a summary of the mortality rates in Table 1. As you can see, we have combined the data from the first two experiments. Of particular interest is the fact that the mixture killed 80% of the monkeys, 32% of the rats and 99% of the mice. It is important to note that here again two monkeys survived the 90-day exposure in the second run, as they did in the first, in remarkably good condition, and none of the 16 monkeys succumbed from a common cause. Some had CNS lesions, some had kidney or liver changes and most showed inflammatory reaction in the lung tissue, plus mild to moderate edema. Of further interest was the finding of hematological changes in animals exposed to indole. The chief characteristic of this anomaly was the appearance of numerous, small 1-2 micron granules found in the red blood cells. These granules have been tentatively identified as Heinz Bodies. Mice and rats were most affected in this respect, and no Heinz Body formation was found in monkeys until after the 70th day. Heinz Body formation has been described by other investigators using other compounds, but none to my knowledge has theorized or proven a causal relationship between contaminant and Heinz Body formation, nor has anyone come to any conclusions as to the physiological implications of this phenomenon. Suffice it to say, however, that it would be a strong evidence for serious consideration of rejection during materials selection.

Carbon tetrachloride, while not causing death at 25 ppm, did cause serious clinical and microscopic liver changes in all animals exposed. The livers of the rats were so much involved that the pathologist made a diagnosis of "cirrhosis". Phenol caused absolutely no problems whatsoever at the 5 ppm level.

Hydrogen sulfide (20 ppm) did produce death in rats and mice, but none in monkeys. Methyl mercaptan (50 ppm) caused serious problems and death in 40% of the monkeys and 43% of the mice. As you will remember, I stated before that engineers, five years ago, would allow 50 ppm of this compound in space cabin atmospheres. It is quite obvious that animals, at least, won't tolerate this concentration during continuous exposure. As an additional experiment, a number of mice were exposed to 50 ppm methyl mercaptan and 20 ppm hydrogen sulfide, with the result that a significantly larger number of animals died than could be accounted for when either of the compounds was administered alone. This experiment shows the possibility of interaction, synergism, or at least additive effects, when mixtures of compounds are used.

We were quite interested in finding out whether animals could perform strenuous tasks following the 90-day exposure. In a sense, we wished to find out whether the chemical stresses involved would seriously affect physiological reserve such as might be experienced during the re-entry phase of a space mission. To this purpose, a swim test was utilized as outlined roughly in a paper by Wilber and modified by Sandage. In essence, the animals were put in a tank filled with water and required to swim until exhausted. This test showed that animals receiving phenol swam significantly longer than their control counterparts. On the other hand, those receiving other compounds were either unchanged or only slightly decreased in swim-time ability. There was no consistency between animals receiving the same compound or between groups, making the evaluation of these data difficult.

Next, we decided to compare the long term, continuous toxicity of some propellants and propellant types in which the Air Force has an interest. We selected hydrazine, UDMH, nitrogen dioxide and decaborane as our candidate materials. For these experiments we changed the design of our exposure chambers, since we were worried about the flammability of the fuels and also the containment problems inherent to our walk-in chambers. As you can well guess, at this time we did not know much about the human tolerances to these compounds, and we had to think in terms of protecting personnel working around these chambers. Even though we were using Threshold Limit Values, there was always the danger of leaks or spills. It is a good thing that these precautions and changes in construction were accomplished because during the first week of exposure the decaborane caught fire and all experiments had to be started over because of possible contamination between chambers. The chambers were made of stainless steel, all were 6 feet tall, and 6 feet in diameter. The animal cages were placed within the chambers in a "lazy-susan" configuration and stacked in 3 layers with mouse cages on the top, rat cages in the middle and monkey cages on the bottom. For purposes of feeding, watering and obtaining biological samples, the chambers were entered through a square door made of 0.25" lucite framed with wood and sheeted on the inside with aluminum. The fronts of all 4 chambers were sealed into one side wall of an anteroom which was built to provide a service area during the experiment. Also, in the event of an accident, this room could supply "clean" air for the animals. The posterior side of the chambers housed the contaminant feed and control systems. The volume of air delivered into the chambers was 25 cfm for hydrazine, decaborane and NO_2 , and 37 cfm for UDMH. The difference of airflow in the UDMH chamber was accounted for by the needs of the induction system. Uniform concentrations of each chemical were maintained by metering carrier nitrogen gas through flow meters and separately controlling the temperatures of contaminant reservoir and mixing chambers with thermostatically-regulated heating mantles. Safety devices on the induction system shut off the carrier gas flow in the event of power failure. Sampling systems were placed in different areas of the chambers, and grab samples were taken at least three

times a day in order to prove breathing zone concentrations. This low concentration work was extremely difficult because of the chemical properties of hydrazine and UDMH, and though we strove for 1 ppm hydrazine, 0.5 ppm UDMH, 0.05 ppm decaborane and 5 ppm nitrogen dioxide concentrations, we ended up with averages of 0.78, 0.56, 0.047 and 4.5 ppm concentrations, respectively. One must also realize that none of these experiments were truly uninterrupted, since doors had to be opened for feeding, watering and biological specimen retrieval purposes. This meant that chamber doors had to be opened for 15-30 minutes per day for cleaning, etc., and three times during the three months the animals were removed for blood collection. So we didn't have a truly uninterrupted exposure as we do with the "Thomas Domes".

Returning to the summary of mortality rates, we can see in Table 2 that hydrazine was the most toxic agent for mice and rats. All mice died within the first 4 weeks; and all but 2 rats died within 10 weeks. Monkeys appeared to be more tolerant; one died in the 4th week, and the second died during the last week of the 90-day exposure. Decaborane, a highly toxic compound, exhibited less species difference. Six monkeys died between the 3rd and 8th week; 23/25 deaths in rats occurred during the first 8 weeks; and 66/82 deaths in mice occurred during the first 3 weeks. UDMH was better tolerated than either hydrazine or decaborane since only 1 monkey, 3 rats and 6 mice died during the experiment. The single monkey's death occurred around the midpoint in the exposure, with 3 rats dying during the second half of the exposure, and mouse deaths were restricted to the first half. Nitrogen dioxide, with no deaths, was the best tolerated agent in monkeys. In rats, the 9 deaths occurred during the first half of the exposure, while the 13 mouse deaths were randomly distributed. Table 3 will show the terminal swim time experiments, conducted to estimate the physiological reserve of the exposed and control animals. All surviving monkeys were able to swim as long as their controls. While all rats died from hydrazine before they could be tested, those that survived the UDMH and decaborane exposures were exhausted in significantly shorter time than the controls. There was very little impairment of swim time with NO₂. Mice showed an average decrease in swim time with all propellants. Growth rate in the rodent was significantly affected by decaborane, and the most severe weight loss was produced in monkeys by hydrazine.

I should like to give a little of the detail of the pathological changes seen during this study. All 3 species exposed to hydrazine showed various gross pathological effects which included fatty changes in the liver. Renal and myocardial involvement were seen infrequently. This is somewhat in contrast to the data which Dr. Patrick and I found during daily injections of varying amounts of hydrazine since we did see marked renal and myocardial

effects. With UDMH, liver degeneration was observed in the monkeys, whereas kidney and heart lesions were predominant in rats; and the liver of mice showed brown pigmentation -- probably hemosiderin. Dr. Patrick's data did not show marked liver changes in monkeys with UDMH when given daily for 20 days. Decaborane produced fatty changes in liver and an occasional involvement in kidney and heart. The most marked pulmonary lesions among all 4 propellants studied were found in the rats exposed to decaborane. The lesions included bronchiectatic bronchopneumonia, chronic peribronchitis with or without bronchiectasis and bronchitis, and eosinophilic alveolar membranes. Interstitial nephritis was a common finding in the rat, and lung involvement was prevalent in mice. This was possibly not compound induced. With nitrogen dioxide, there were minimal pathological changes. All these changes were found in contrast to relatively small changes in the clinical chemistries which were performed throughout the experiment.

I would now like to take the remaining time to discuss the implications of our first sojourn into long term continuous inhalation exposure experiments and to bring out some possible philosophical ramifications surrounding this type of work. Perhaps I can also provoke some spirited discussions during our round table panel sessions tomorrow. First of all, these experiments have proven without doubt that industrial TLV's cannot be used to extrapolate long term exposure criteria, since our data indicate no predictable relationship between TLV and potential toxicity during long term exposure. For example, phenol has a TLV which has such a great built-in safety factor that its use in these experiments produced no ill effects whatsoever. In fact, one could believe that it's actually beneficial in these experiments since our swim tests indicated that they were more resistant after exposure! Further, in the case of UDMH and hydrazine, we found that although hydrazine has a two-fold greater TLV than UDMH, these experiments proved it to be a much more dangerous compound than UDMH.

Secondly, we have still not found the correct test for evaluating overall chemical stress. Although we did find some changes utilizing the swimming stress test, it was quite obvious that the changes were highly suspect, and slight changes in procedure evoked different responses. For instance, very slight differences in water temperature directly affected the length of time which an animal could swim. Besides this, it seems to make a real difference between species as to what temperature the water must be for optimum conditions. Water temperature for monkeys was optimum at 22°C, while 17°C was best for rats and mice. In addition, using this type of test, we ran into some problems during gross and microscopic examination of lung and heart tissue, and I have serious doubts that some of the clinical chemistries after strenuous exercise would not change. In our laboratories we have recently shown changes in certain isoenzymes following heat stress and strong exercise. Needless to say, I believe the swimming test to be poor for studying physiological reserve. We are currently getting

ready to perform psychopharmacological performance testing on our animals before, during and following long term exposure. We have hopes that this type of evaluation will be refined enough to pick up more subtle changes in performance and hence be an early indicator of chemical stress. In conjunction with this, as I have mentioned before, we are striving to compare certain enzyme and isoenzyme patterns of blood as early indications of chemical stress. We are also currently looking at radiorespirometry, utilizing labeled sugars, as a possible method for detecting early physiological changes. Also, as a future project, we are looking forward to the time when we can utilize our new acceleration facility here at WPAFB. In this instance, following long term continuous exposure, we could then subject some of our animals to the kind of G-force stresses that might be encountered during the re-entry phase of an orbital spacecraft. Perhaps as time goes on, we can combine a number of these tests to provide the kind of factual evaluation that we will need in our future manned space missions. We would welcome ideas along these lines from this audience.

These are indeed really exciting times, and we have just barely scratched the surface of possibilities for methods of providing tolerance criteria to space cabin materials. There may be a number of different "fringe benefits" from the kind of research which we are now doing. One might obtain data which will aid in interpreting air pollution; or one might find new ways of speeding up studies on the chronic toxicity of drugs by utilizing continuous exposure techniques. We have already gained some bonuses by increasing knowledge of some of the clinical laboratory aspects of the dog and the monkey. As you have heard, we are doing a great deal of work which will ultimately give us some statistically valid historical controls for large laboratory animals. Much of the data in the literature has been erroneous, vague and very often performed on too few animals for statistical evaluation. These data compiled by Capt. Siegel, Navy Toxicology Unit, the Air Force and various other laboratories should be sufficient to provide the scientific community with this much needed information. At any rate, I think that during the discussions of these two days, we will all arrive at a better appreciation of the problems encountered with equipment, animals, the discrepancies in clinical chemistries, the problems in obtaining consistent breathing zone samples, the problems in identifying analytically trace contaminants, the differences in species response to the same agent, and last, but certainly not least, the very real problems of interpreting our data in the light of differences obtained from all our contractors and colleagues working toward the same goals.

TABLE I
SUMMARY OF MORTALITY RATES

COMPOUNDS*	MONKEYS		RATS		MICE	
	# DEAD # USED	% DEAD	# DEAD # USED	% DEAD	# DEAD # USED	% DEAD
N ₂ H ₄ UDMH NO ₂ BiOH ₁₄	2/10	20	48/50	96	98/100	98
	1/10	10	3/50	6	6/100	6
	0/10	0	9/50	18	13/100	13
	6/10	60	25/50	50	82/100	82
	1/10	10	0/50	0	1/100	1
CONTROLS						
CCl ₄ PHENOL INDOLE H ₂ S Me•SH MIXTURE**	1/10	10	0/50	0	0/100	0
	0/10	0	0/50	0	0/100	0
	2/10	20	5/50	10	22/100	22
	0/10	0	12/50	24	26/100	26
	4/10	40	5/50	10	43/100	43
MIXTURE**	16/20	80	32/50	64	99/100	99
CONTROLS	0/19	0	2/50	4	38/200	19

*AT TLV CONCENTRATIONS

**INDOLE, H₂S, Me•SH, AND SKATOLE

TABLE 2

TIME vs MORTALITY

DEAD
USED

	2	4	6	8	10	12 WEEKS
N_2H_4	<div>98</div> <div> <div>98/100 MICE</div> <div>48/50 RATS</div> <div>2/10 Mo.</div> </div> <div> <div>46</div> <div> <div>98/100 MICE</div> <div>48/50 RATS</div> <div>2/10 Mo.</div> </div> </div>					
$B_{10}H_{14}$	<div>66</div> <div> <div>82/100 MICE</div> <div>25/50 RATS</div> <div>6/10 Mo.</div> </div> <div> <div>16</div> <div> <div>82/100 MICE</div> <div>25/50 RATS</div> <div>6/10 Mo.</div> </div> </div>					
UDMH	<div>X</div> <div> <div>6/100 MICE</div> <div>3/50 RATS</div> <div>1/10 Mo.</div> </div> <div> <div>X</div> <div> <div>6/100 MICE</div> <div>3/50 RATS</div> <div>1/10 Mo.</div> </div> </div>					
NO_2	<div>X</div> <div> <div>13/100 MICE</div> <div>9/50 RATS</div> <div>0/10 Mo.</div> </div> <div> <div>X</div> <div> <div>13/100 MICE</div> <div>9/50 RATS</div> <div>0/10 Mo.</div> </div> </div>					
INDOLE + H_2S + ME·SH + SKATOLE }	<div>87</div> <div> <div>99/100 MICE</div> <div>52/50 RATS</div> <div>16/20 Mo.</div> </div> <div> <div>10</div> <div> <div>99/100 MICE</div> <div>52/50 RATS</div> <div>16/20 Mo.</div> </div> </div>					

TABLE 3
SWIM TIME (MINUTES) OF MONKEYS, RATS AND
MICE EXPOSED TO VARIOUS AGENTS

<u>Agents</u>	<u>Monkeys</u>		<u>Rats</u>		<u>Mice</u>	
	<u>Average</u>	<u>Range</u>	<u>Average</u>	<u>Range</u>	<u>Average</u>	<u>Range</u>
Control	26	18-31	41	23-100	39	7-99
Hydrazine	21	11-34	Died		Died	
UDMH	26	20-34	21	14-39	11	3-17
Decaborane	21	14-31	21	9-33	12	7-19
Nitrogen Dioxide	27	19-30	34	29-43	20	13-45

REVIEW OF AMBIENT PRESSURE ANIMAL EXPOSURE DATA FROM SELECTED NAVY COMPOUNDS

By

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Before delving into the material studied in our Unit, it might be worthwhile to review briefly the objectives, method of approach and to some extent, our philosophy. The task assigned to the Navy Toxicology Unit is shown in Table 1. I show this to give an overall picture that we are an operational unit, not a research unit, and I will spend most of my time, practically all the rest of the time on the Steps of Task #1, on the Long Term Chronic Inhalation Studies. Our primary objective has been to provide rapid, practical answers to the forces afloat in the area of toxicology. Our studies have been oriented toward operational requirements in a resolution of existing or anticipated problems, although basic research aspects are included in our mission. For example, there was, and still is, a constant need to search for better methods of contaminant generations, for more reliable methods of analysis and monitoring, for new bio-chemical predictors, and for new ways of getting more information from the exposed animal. Many of these items were discussed this morning, and I will just touch on a few of them.

About five years ago we started intensive work in relation to the Polaris program. We froze on the Rochester-type chambers developed by Dr. Hodge and his group for the Atomic Energy Commission, as being the best design for our purpose. Some minor modifications were made and these are shown on Table 2. I didn't hear any mention about aerosols this morning so I will talk about them for a moment. We have an arrangement by which we try to maintain our maximum particle size at about 2-1/2 microns. The low end we don't know, but we do know we have nothing above 5 microns. Step #2 is similar to what they've done here. We do open our chamber for feeding, and in that sense it is not a continuous exposure, but we do have a shower ring installed in there so that we can clean out intermediately and not have to open it as much as we would otherwise.

Each of our chambers is equipped with electrostatic precipitators so that we can clean out dust and oil mists before discharging them to the atmosphere.

A little schematic of our chamber -- (Figure 1) they're usually in pairs. We have six of these chambers and the test material goes in through what we call a mushroom impactor or a mist generator where the particle size is

controlled. It goes into the chamber, mixes with the air-conditioned air. The animals are exposed. It goes through the electrostatic precipitator, through various filters and charcoal so that the air going out is clean. With this setup we have exposed animals, usually dogs, monkeys, rabbits, rats and guinea pigs, to a number of chemicals of military interest. Although we were initially solely interested in long-term continuous exposure as might be expected aboard a submarine, it quickly became apparent that comparable data for industrial type of exposure, 8 hours per day, 5 days per week, with the weekend off, would be useful for the following reasons: It is known that exposures at Navy shore establishments and aboard surface vessels are similar to that in industry. Secondly, the 30-day repeated exposure study may help supply additional documentation for some of the existing TLV values; and, finally, it would appear useful to build up a body of data that could be compared. For example, 90 days continuous exposure versus 30 days repeated exposure.

These are the animals (Table 3) that we have used in all of our studies, except on triarylphosphates where we have substituted chickens for rats and other rodents. The point I'm trying to make here is that we are satisfied with our guinea pigs. They are raised by the Naval Medical Research Institute. The rats, we started off with Long-Evans and they were very fine animals. No murine pneumonia, but NMRI decided to stop breeding them and we had to go to Sprague-Dawley and we have plenty of troubles with them now. Rabbits we buy from open purchase -- and they are terrible. The monkeys are lousy. The dogs are very good Beagle dogs that are bred for the Navy.

A word about our controls. For economic reasons it is not feasible for us to run a control chamber for each experiment. In the earlier experiments, animals exposed to low levels of various contaminants where no ill effects were noted served as controls. Although not a pure control, this represented animals exposed to similar stress conditions of confinement, temperature, humidity and airflow. During the past two years, control animals have been maintained in an exposure chamber for 90 days and served as controls for the other five experimental chambers. Here, too, there is a problem, because the five exposed chambers do not all go on stream at the same time and control animals are not litter mates.

Table 4 is a summary of our control over a period of four years, the mortality percent you can see is not too bad. We run maybe three rabbits in a chamber, or two sometimes. One rabbit will give us 50% and the same problem with the monkeys where we only run three monkeys in a chamber. But I would like to point out that the guinea pig 0-7% we would accept as a normal control pattern. It would have to be above that before we would be satisfied that there was any change in the animal. The same with the rats. There would have to be better than 13%.

Table 5 shows the parameters that we've studied. In hematology we've studied hemoglobin, hematocrit, WBC and differential, and in biochemistry we've studied protein fractions, both chemical and electrophoretic. We've done Warberg-tissue-oxygen uptake, kidney function tests, liver function tests, methemoglobin formation, RBC fragility. Under enzymes we have studied SGOT, SGPT, isocitric dehydrogenase, alkaline phosphatases, lactic dehydrogenase, anaerobic lactic acid production utilizing kidney and liver tissues. Previous enzymes were in serum. Now of all the enzymes that we have studied, the only one that seems to give promise at the present time is isocitric dehydrogenase, and although the anaerobic lactic acid production showed promise in the beginning, we have abandoned it at this time.

Now, this may sound different from the results reported by the Wright-Patterson people this afternoon, but I want to call your attention to the fact that the levels we run are really trace contaminant levels and in the order of approximately 100 times, 200 times less than what was reported here at Wright-Patterson, so that those levels, those changes should not be compared. The exposures were at a much higher level than we have run here. Although we have done pathology, organ weight, hematology, body weight, biochemistry, enzyme studies and watched for signs of ill health, we are still in a primitive stage since we have depended mostly on a mortality picture. Here we are getting some help from Dr. Coulston and his group at Albany who are studying the materials down at the cellular level.

Table 6 shows a group of materials that we've studied. The "C" is a continuous 90-day exposure. The "R" is a 30-day repeated exposure, 8 hours per day, 5 days per week. Under the oxidants, we've studied ozone, nitrogen dioxide, chlorinated hydrocarbons, carbon tetrachloride, trichlorethylene, freon, methyl chloroform, vanillidin chloride, mixed hydrocarbons, hydraulic oil - 2190, paint thinner, and a synergistic run of paint thinner and cumene. Under aromatic hydrocarbons, benzol, toluol, orthoxylol and cumene, and organic phosphates, we tried triarylphosphate, hydraulic fluid group and tri-orthocresyl phosphate. Under miscellaneous we've covered ammonia, dimethylamine, formaldehyde, ethylene glycol, ethanol, and under classified, for those people who might be interested, is MCS-198, a Gemini coolant and PR=155 an ordnance material.

Table 7 tabulates the mortality for the five species of animals for NO₂ and ozone, and if you'll notice under NO₂, 123 milligrams per cubic meter, one-hour exposure was enough to practically wipe out our exposed animals. This is somewhat different, I think, from what is happening in the report that was presented here from Wright-Patterson, and I believe that this is one of the reasons why we're having a meeting of this sort, to try to resolve where the differences are. At 68 mg/m³, which is a little bit lower than what they showed, we still would have a very high mortality. Then under the "C's" we have the continuous 90-day runs which are these three columns for NO₂. There is some orderliness, except when you get to the damned rabbits again. Otherwise, there seems to be some dose response relationship.

Figure 2 shows the mortality curves. We don't have many points there on the ozone. The number of runs for nitrogen dioxide are indicated, and the dotted line is not a true part of the curve, but at the lower level we get no effect.

I've elected in making this presentation just to take two groups of material. The first one was the oxidants, now the next is a hydrocarbon, to demonstrate what we're getting, although I might preface it by saying that we're getting no pathology. We are practically getting negative results all the way through except mortality, and that's why we're using mortality.

Now, we spent quite a bit of time on paint thinner because it's a real problem aboard the submarines for various reasons. Here we find our best picture in the guinea pigs where we have somewhat of a dose response (Figure 3).

In this graph here we have a fair picture (although what you'd want is a nice straight line). I have never seen a good straight line yet in this kind of work; and, in fact, we never have run a good experiment yet. This gives you a picture of what we have found.

Now, I could go into the composition of the paint thinner, for those who might want it. I'm not going to spend much time on it, but I will tell you that it contains roughly 85% saturated hydrocarbons, 1.2% olefins and approximately 13% aromatics. I think the aromatics are what are of concern to us.

On the basis of this plus other work that we've done, we have set a 90-day level for continuous exposure at 40 milligrams per cubic meter, which is pretty low. If the aromatic content should be higher, we would probably have to drop it even lower than that. I mean, this is open argument whether this is a good extrapolation or not, but this is what we've done. We thought we gave ourselves, well, maybe a factor of safety of only 5 to 10 on this thing.

Now, paint thinner and cumene should have been at least additive, we thought, and we got a lower percentage of mortality in the guinea pigs. Why? I don't know, but we're going to repeat it.

At the request of Dr. Thomas, I've prepared a few tables to show some comparative data on the materials that have been done at Wright-Patterson.

Table 8 shows accumulative data based on carbon tetrachloride for 30-, 60-, 90-days exposure. The question is, can you stop at 30 days or can you stop at 14 days. Our impressions, based on the guinea pig -- that you can't get enough data. The longer you carry it out at these low levels, the more effect you get.

Table 9 is on ozone and we've got three levels here; and, again, remember, these levels are far lower than levels run at Wright-Patterson by a great

amount. You take 1.4 level, you get some picture from 30 to 60 to 90 days, and at .34 level, the same way. If you go down lower, you get nothing, so if you're using real, real low concentrations, it doesn't make much difference whether you stop on the first day or the 90th day, but if you have something that may have an effect, then you'd better continue that for a reasonable period of time. 90 days may not be long enough to get the picture.

Table 10 is on nitrogen dioxide. Here again the rats show somewhat of an increase; the guinea pigs have a definite order. We have, I think, a true effect in the monkey at the higher level, and at the other levels, apparently nothing. The dogs have been very resistant all the way through.

Table 11 compares three laboratories: Midwest Research Institute, our own and Freeman. Theoretically, all three used the same type of rats. The number of animals varies, the % mortality varies in this case -- 20% and 0 and 0. Yet the duration is essentially the same. How to get these things to jibe, I think, is another point for this group to take up and try to resolve.

Now, future plans for the Navy Toxicology Unit are summarized in Table 12. We now have equipment where we are in a position to telemeter three functions, ECG, brainwaves and respiration rate. This is presently being checked out, but not yet in animals. It's being checked out on a simulated run. Our problem has been that many animals have died with no pathology either gross or histology. No signs or symptoms or whatever you want to call them, and yet they're deadlier than hell. We're hoping possibly to get some information by telemetering. Less blood work. We feel that if we do work at the beginning and at the end without disturbing the animals in the middle, we feel this is an unnecessary stress, it takes them days to recover, that we will be better off and get a better picture. Occasionally we have lost an animal which we think was due to handling or shock or whatever it was, and other people have reported this in the literature. I think we'd be much better off in the long run not to fool with the blood work in the middle of the run and not hurt the total picture, unless you can do it without molesting the animal. Now the rabbits, I've said enough about that.

We already have an engineering study on -- the Navy is going into the deep sea submergence program, and a chamber is now being engineered for us which will have a number of features. It's going to be a rough one because we expect to go down 2,000 feet in the chamber, and you're not going to be able to put people in there. It's going to be an animal chamber for a long, long time, so we think we're going to have to have automatic and continuous cleaning of the chamber, automatic feeding, capability from the AEC to remove carcasses of dead animals through a lock, TV viewing of animals, telemetering, and I think we're going to have a problem introducing the contaminant when we get into the very, very high pressures. We hope to do more synergistic studies. We've done just one and we're just scratching the surface. We are planning a new building for the Toxicology Unit, to be out at the same

medical center at Bethesda. This will be 22,000 feet and, in addition to pressure work on animals and ambient and so on, we will stress human exposures because we think we have to go to that in order to get the answer. We don't think we can talk to the animals enough and get them to tell us about their feelings. We're going to have to go to humans in order to get the picture, and I think at that time we will also do psychological measurements of degradation of performance and tag the materials with isotopes so that we can get some handle on what's happening.

TABLE 1. TASKS

1. Conduct long-term chronic inhalation studies on military chemicals.
2. Conduct short-term acute toxicity studies.
3. Evaluate air contaminants aboard ship.
4. Develop toxicity data needed to set limits.
5. Evaluate material, equipment, and processes which may generate potentially toxic materials.
6. Evaluate field equipment and instruments for measuring air contaminants.

TABLE 2. CHAMBER MODIFICATIONS

1. Particle size of aerosols is controlled.
2. Air samples are taken at fixed points.
3. Shower-ring for interim cleaning without opening chamber.
4. Electrostatic precipitator in exhaust line.
5. A 9 channel alarm and recording system for each chamber.

TABLE 3. ANIMALS USED

Guinea pigs - Hartley (NMRI colony)
 Rats - Long Evans and Sprague Dawley (NMRI colony)
 Rabbits - New Zealand Albino (open purchase)
 Monkeys - Squirrel (open purchase)
 Dogs - Beagle (REEL - Navy)

TABLE 4. INHALATION STUDIES USED AS CONTROLS
(10/3/60 to 12/1/64)

	<u>Rat</u>	<u>GP</u>	<u>Rabbit</u>	<u>Dog</u>	<u>Monkey</u>
Mortality	16/641	3/493	2/98	0/66	2/98
Mortality %	2.5	0.60	2.0	0	2.0
Range %	0-13	0-7	0-50	0	0-33

TABLE 5. PARAMETERS STUDIED

Visual signs	Mortalities
Body weight	Gross pathology
Hematology	Histopathology
Biochemistry	Organ weight
Enzymes	Bone marrow

TABLE 6. CLASSES OF MATERIALS STUDIED

	No. <u>Materials</u>	No. of runs	
		<u>90 day C</u>	<u>30 day R</u>
Oxidants	2	8	3
Chlor. hydrocarbons	5	5	2
Mixed hydrocarbons	3	13	4
Aro. hydrocarbons	4	7	4
Organic phosphates	2	9	3
Miscellaneous	5	9	4
Classified	2	5	3

C-Continuous R-30 exposures, 8 hrs/day, 5 days/wk

TABLE 7. OXIDANTS

	Conc mg/m ³	Type Study	Mortality Percent				
			Rat	GP	Rabbit	Dog	Monkey
NO ₂	123	*	40	87	33	100	100
TLV 9 mg/m ³	68	R	27	53	66	0	100
	22	C	37	63	17	0	33
	8.3	C	0	13	66	0	0
	1.0	C	7	0	0	0	0
Ozone	2.0	R	0	0	66	0	0
TLV 0.2 mg/m ³	1.4	C	49	97	67	0	0
	0.34	C	0	57	0	0	33
	0.13	C	0	7	0	0	0

* One 8 hr exposure C-Continuous R-30 Exposures
8 hrs/day, 5 days/week

TABLE 8. COMPARISON OF 30, 60 AND 90 DAY MORTALITY
Carbon Tetrachloride - TLV 65 mg/m³

Conc mg/m ³	Days	Percent Mortality, Cumulative				
		Rat	GP	Rabbit	Dog	Monkey
60	30	0	0	0	0	0
	60	0	7	0	0	0
	90	0	20	0	0	0

TABLE 9. COMPARISON OF 30, 60 AND 90 DAY MORTALITY
Ozone - TLV 0.2 mg/m³

Conc mg/m ³	Days	<u>Percent Mortality, Cumulative</u>				
		Rat	GP	Rabbit	Dog	Monkey
1.4	30	0	43	66	0	0
	60	7	93	66	0	0
	90	47	97	66	0	0
0.34	30	0	20	0	0	0
	60	0	43	0	0	0
	90	0	57	0	0	33
0.13	30	0	0	0	0	0
	60	0	0	0	0	0
	90	0	7	0	0	0

TABLE 10. COMPARISON OF 30, 60 AND 90 DAY MORTALITY
Nitrogen Dioxide - TLV 9 mg/m³

Conc mg/m ³	Days	<u>Percent Mortality, Cumulative</u>				
		Rat	GP	Rabbit	Dog	Monkey
"22"	30	17	7	0	0	17
	60	33	43	0	0	17
	90	37	63	17	0	33
8.3	30	0	0	33	0	0
	60	0	13	33	0	0
	90	0	13	66	0	0
1.0	30	0	0	0	0	0
	60	0	0	0	0	0
	90	3	0	0	0	0

TABLE 11. NITROGEN DIOXIDE

Sprague Dawley rats

	<u>Conc ppm</u>	<u>Mortality percent</u>	<u>No. Animals used</u>	<u>Duration of exposure</u>
MRI - AF	4.5	20	41	90 days
NTU	4.4	0	15	90 days
FREEMAN	4.0	0	9	114 days

TABLE 12. FUTURE PLANS

Telemetrying

Less blood work

Discard rabbits

High pressure chamber

Synergistic studies

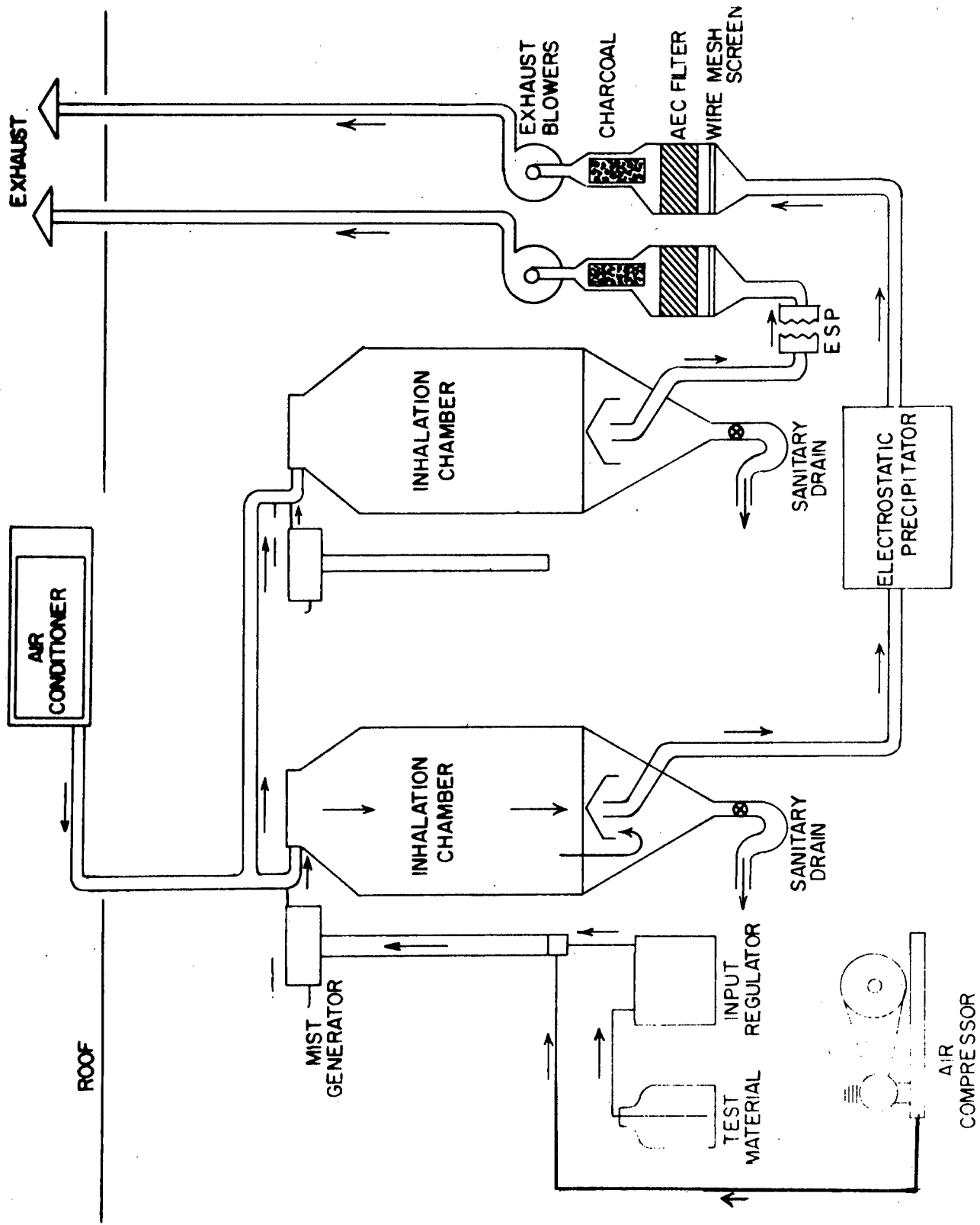


FIGURE 1.
 SCHEMATIC - ONE PAIR OF INHALATION CHAMBERS U.S. NAVY TOXICOLOGY UNIT

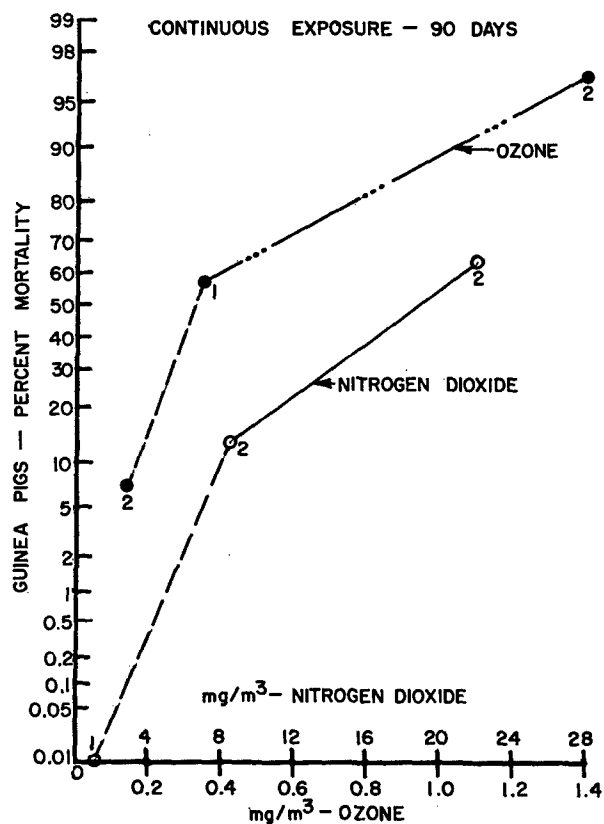


FIGURE 2.

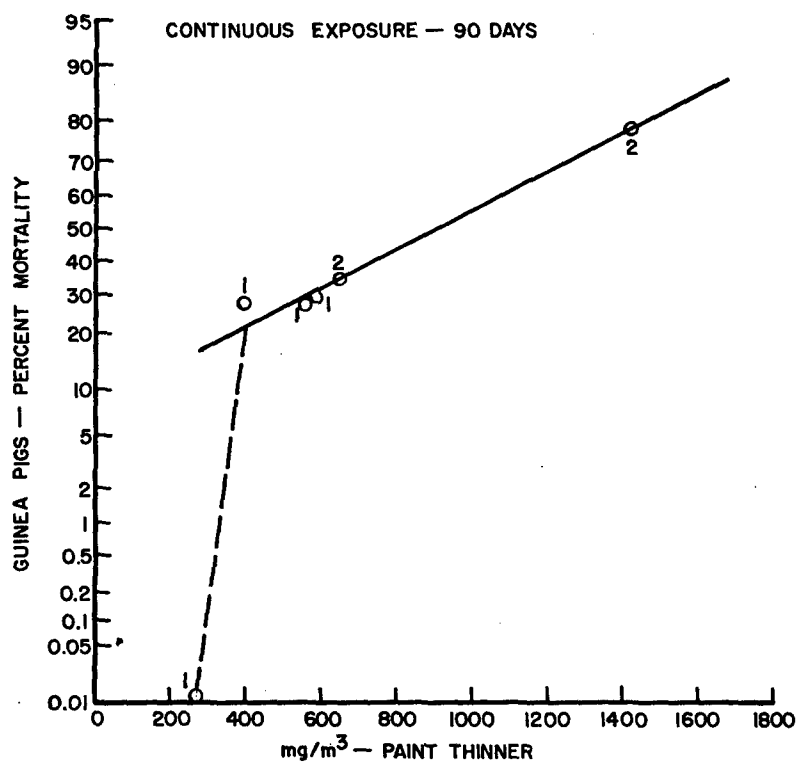


FIGURE 3.

LONG TERM INHALATION EXPOSURE EXPERIENCE WITH REFERENCE TO AIR POLLUTION

By

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As part of the overall program by the Division of Air Pollution, our laboratory, the Laboratory of Medical and Biological Sciences, has been charged with studying the biological effects on plants and animals, including man, of chronic exposures, long term exposures to air pollution as it exists in the ambient atmosphere of communities. So we have been concentrating on mixtures of normal air pollution, not single agents or simple mixtures of pure gases. Our initial studies which I am discussing today primarily are concerned with chronic exposure to oil exhaust, contaminated atmosphere, both raw oil exhaust as it comes from the tail pipe, as well as irradiated oil exhaust which simulates the photochemistry that occurs due to sunlight causing the auto exhaust to react.

In the basement of our facility we have an engine dynamometer room (figure 1) which contains two standard six-cylinder auto engines, one is a standby, which are connected through an automatic transmission to an engine dynamometer which puts the same load on the engine as your car would. These engines are cycled through automatic controls so that they proceed through acceleration, cruise, deceleration, acceleration, etc. The exhaust produced by the engines is then diluted with clean air. This is air that has been passed through particular filters as well as CBR activated charcoal and we have four separate concentrations of both the raw and irradiated atmospheres. After it has been diluted to the desired concentration, it is split, and half of it is taken immediately up to the animal exposure room and supplied to the raw exhaust exposure chamber. The other half passes through 680 foot of irradiation chamber which has light banks on both sides which simulate sunlight and are allowed to undergo photochemical reactions. The life of a volume of gas in these chambers is approximately 62 minutes. Following the emission from the chamber it is piped up to the irradiated exhaust exposure chamber containing experimental animals.

With this particular experiment, we had four concentrations of raw and four concentrations of irradiated, as well as 16 clean air chambers for control animals.

Not only was the exhaust produced and supplied to the animals as raw and irradiated, but it was produced and supplied in a cyclic manner, in order

to simulate the diurnal variations in the various components of the exhaust that occur in the urban-type atmosphere.

As a result of this diurnal fluctuation in components, figure 2 indicates the peak control of concentrations that we attempted to attain. We automatically and continuously monitored the carbon monoxide, NO, NO₂, hydrocarbon which is expressed here as hexane, and ozone. This, of course, represents irradiated auto exhaust. I might also say here that when raw exhaust undergoes irradiation, NO₂ is produced as well as ozone, at the expense of NO, and some hydrocarbons enter into the reaction. So you can see the peak control of concentration is right approximately as you see them here with concentrations increasing from 1 to 4.

Now the diurnal picture is indicated on figure 3 for the irradiated exhaust atmospheres. This contains all the major contaminants I just indicated. At approximately 8:00 in the morning, our engines were turned on and certain buildup time or equilibration time in the irradiation chambers had to occur, of course, then the irradiation lights were turned on at approximately 10:00 a.m. At this time you will notice, for example, the NO decreases in concentration because it is being used up in the photochemical process. NO₂ would be produced and peak, and ozone would begin to be produced and again peak at a later time.

In addition to this, some secondary photochemistry occurred which is not indicated on here resulting in production of oxidants.

Then at about 3:30 p.m. the engine was turned off and the exponential die-off from the chambers was allowed to occur until about 25% of the peak values occurred which we refer to as "trough" values. This was maintained until 8:00 o'clock at night when the engine dilution was decreased and they were allowed to peak again, but without photochemistry, that is without irradiation light. So these animals received irradiated exhaust such as you might have in a city where the peak traffic pattern occurs, you have sunlight and photochemistry; when the evening traffic pattern occurs you do not have sunlight, so you do not have photochemistry. A comparable diurnal cycling of components in raw (non-irradiated) exhaust diagrammatic is presented here (figure 4). Of course, when the engine was cut off, since there was no dilution chamber between the raw exhaust and the exposure chamber, you don't have this nice curve effect, you just have a real fast buildup, a dropoff and etc.

The total concentration time exposures to components that are common to both raw and irradiated, such as carbon monoxide, or total hydrocarbons, would come out about the same in the end over a chronic, long time exposure.

The values that you saw on figure 2, peak and trough values, are values obtained at the header as it comes into the animal exposure room. Actually,

when one passes this material through the exposure chambers containing the experimental animals, due to the presence of organic matter, the main organic matter being the experimental animals themselves, there is a marked reduction in some of the more reactive components. We would monitor periodically the concentrations going into and out of randomly sampled exposure chambers, and we routinely observed in our old chambers 80 to 85% loss in ozone; 8 to 12% loss in NO and a 45 to 55% loss in NO₂. So, actually the concentration the animals were inhaling was not as marked as the table would indicate.

The automatic instrumentation -- we used an infrared analyzer for carbon monoxide and an hydrogen flame ionization detector for hydrocarbons. A coulometric oxidant sensor using buffered potassium iodide for oxidant, and an NO-NO₂ continuous recording colorimetric analyzer employing a modified Saltzman reagent. Periodically, we used also wet chemistry for formaldehyde and acrolein.

There are nine atmosphere treatments (four levels of four raw, four irradiated and one pure air control). Each of the irradiated and raw concentrations was furnished to ten exposure chambers, and clean air to 16 chambers and each of these chambers received 15 atmospheric changes per hour. The limiting factor here being the amount of irradiated exhaust which could be obtained from our irradiation chambers.

A certain amount of time was required to produce the photochemistry, and this limited the amount of air changes we could have in any chamber of the experiment.

Now, per treatment atmosphere, the experimental animals that we utilized included 32 female Swiss mice; 8 pair of old, that is retired, breeders A_j strain; 8 pair of old C57/B; 24 pair of LAF₁ strain which is really the first generation resulting from the cross of a C57 female with an A_j male; 24 pair of young A_j; and 24 pair of C57 blacks of the same age group; 16 pair of 101 strain; 7 pair of Sprague-Dawley rats; 8 hamsters which were male, Golden Syrian; 3 pair of Hartley guinea pigs and 3 females and 2 males of NIH strain of guinea pigs. This resulted in a total experimental complement then of 2313 experimental animals. So you see, we had a logistics problem to deal with.

These animals were randomly assigned to the replicate exposure chambers for each of the 9 experimental atmospheres. Figure 5 indicates a typical individual treatment design.

In an attempt to reduce additional variables in the experiment, we arranged and allocated the animals so that, for example, animals designated A were used in activity studies. They were always housed with two guinea pigs of comparable strain and four hamsters; whereas animals I, for immunology,

and O for the older mice, were always housed with two rats and one guinea pig; whereas the L for longevity study animals, which were the three young strains of mice I mentioned, are always housed with two rats and one guinea pig. We have sort of a "blocking" effect to try to get away from variables that might come into play by having some of these animals being with hamsters and some of them not, etc.

"P" refers to a "plumbing" chamber which was available on all of our concentration levels for other people in the laboratories who wished to do short time studies.

These atmospheres, by the way, were supplied to our experimental animals seven days a week, including weekends, for periods of up to 11 to 23 months depending upon what strain or species we were referring to.

Figure 6 is a photograph of the standard 6-cylinder auto engine coupled through an automatic transmission to a dynamometer which, as I mentioned, furnishes a comparable load. The exhaust is piped up through a heat exchanger, diluted with pure air to the proper concentration.

Figure 7 is just a picture of the automatic controls for the cycling of the auto engine and for controlling the dilution with pure air and pressures in the chamber.

Figure 8 is a picture of one of our irradiation chambers. We had 4 at that time -- about 680 cubic feet lying on both sides with a bank of lights which simulated the sunlight radiation. Mylar window material. These lights were air cooled. Exhaust was piped in, resided for approximately 62 minutes and then piped out and taken upstairs in as short a route as possible.

Figure 9 shows our automatic instrumentation. This is our NO-NO₂ analyzer. We have a hydrocarbon instrument and a long path infrared and a mast ozone meter. The oxidant sensors were located at the animal exposure chamber table itself so that the oxidant was not transported any further than necessary to cut down on oxidant loss. We actually rigged it so that the electric signal from the mast ozone analyzer was then sent to an 18 point channel recorder.

Figure 10 is a view of half of the animal exposure chamber room. There were 96 total exposure chambers arranged in such a fashion. All the tubing for entrance and exits was teflon tubing. The room housing the animal exposure chambers was also air-conditioned and maintained similar temperature and humidity as the animal exposure chambers themselves. This is about 72°F and about 72% relative humidity.

Figure 11 is a typical cage loading arrangement in the chamber. In this particular chamber, there would be eight mouse cages on the top shelf,

two guinea pig or rat cages used for either species in the middle shelf and eight mouse cages on the bottom shelf.

The exhaust came in through the front corner, passed through a baffle plate and was channeled out the opposite rear corner. Note the ozone analyzer on the bottom of the table.

Figure 12 is a picture of the spontaneous activity cages that we used. The mouse could either live in the little semicircle cage if he liked, he could walk into the activity wheel and sit there if he liked, or he could walk in the run if he liked, so it was purely voluntary. The wheels were hooked up with microswitches and automatically recorded at a central table independent of the rotation of the wheel in terms of revolutions.

Initially, the general biological protocol was designed to be primarily a longevity and carcinogenesis study. As other disciplines came into the picture, other observations were suggested and made. So, in toto, we made observations concerning mortality and morbidity; pathology, both gross and histo, any animal dying in a chamber was sent to a pathology lab. There was a random sample of mice removed from the study after 16 months of exposure. Then, of course, the animals remaining after 23 months were sent to pathology also. We did growth determinations on mice, guinea pigs and rats, in terms of body weight primarily. There was some immunology work done on mice. Reproduction work was done on our very first LAF₁ strain of mouse in terms not only of fertility, but also of survival of the young from birth until weaning at 21 days.

As I mentioned, spontaneous activity was done with our LAF₁ strain mouse predominantly.

Tissue chemistry was performed periodically for some enzyme studies. We usually saw nothing there -- probably because of our very low levels of pollutants. We did do wet and dry lung weights on rats sacrificed out of the study, and we also did routine chemical composition studies on the lungs of these rats. Hematology was done routinely at 8-week intervals and included red blood cell count, using a Coulter Counter, white blood cell count, red cell size distribution studies, hematocrit and hemoglobin as well as blood oxygen, and CO₂ evaluation. Pulmonary function was performed at 16-week intervals on our guinea pigs utilizing a body plethysmograph method. However, I must say that when this was performed on our animals, they had been removed from the polluted atmosphere, and taken to our laboratory where there was essentially clean air. They had a good time for recovery from any atmospheric effects. Our intent was to see if there was a permanent impairment on the pulmonary function of these animals.

It has been shown previously by Dr. Murphy, who was with us at the initiation of this experiment, that there are temporary effects on the animal's

pulmonary physiology which disappear when he is removed from the atmosphere. We could demonstrate no permanent impairment.

In general, there were no statistically significant effects of our auto exhaust atmospheres with respect to mortality, morbidity, growth, immunology, blood gas analysis or oxygen consumption. (I think I failed to mention we did get oxygen consumption routinely on our mice, rats and guinea pigs also.)

As far as pathology is concerned, the only pathology we saw was a treatment correlation in respect to the amount of particulate material contained in the macrophages in the lungs of our mice.

There were significant effects in terms of the susceptibility to infection with increasing age in our mice, and in terms of spontaneous activity. This is interesting in that initially when the animals were placed in either raw or irradiated exhaust atmospheres, they were depressed in activity. The ones on the raw exhaust adapted to the environment and returned to normal (overshooting it, actually) in approximately 12 days. Those on irradiated exhaust required 23 days. After six weeks of this type of exposure, we did a partial switch experiment. We didn't have enough controls to do it with both raw and irradiated, so we did it with irradiated. We took half our controls and put them in irradiated and half of the irradiated and put them in control air, clean air, and, of course, the other half remained where they had been; and, as a result, the animals that had been on clean air and put in irradiated again showed a depression followed by an adaptation. The animals that had been on irradiated and stayed on the irradiated, even though they had adapted initially as you recall, after four weeks of this additional exposure they began to drop off in activity, which suggested to us that we might have been observing an effect in terms of a stress followed by adaptation and then this followed by increased length of exposure and toxicity at the very end. We want to do more work along this line. Again, we might have been observing behavioral effects too.

Mouse fertility data was very interesting. All the animals receiving the two high concentrations of irradiated exhaust were markedly affected in fertility in that there were fewer litters born and, consequently, there were fewer total number of young mice born. The number of potential mothers that could have been was markedly reduced. The frequency of littering per mother that ever had had a litter was markedly reduced, and there was a marked reduction in survival of the young from birth to weaning, particularly during this first five to seven days of life. It did not affect the average number of young mice per litter, nor did it affect the average birth weight of the young mice. We have done some followup work on that and it is presently being written up for publication.

We sacrificed a number of mice from the experiment and performed bone lead analysis on these animals and we could show a trend of increasing bone lead with increasing auto exhaust concentrations. The highest bone lead concentrations we saw, however, were in the neighborhood of 20.5 micrograms per gram of dry bone. The biological significance of that I'm not sure of, I think it is poorly defined.

As far as the lung composition studies were concerned with our rats sacrificed off the study following 11 months of exposure, regardless of whether it was raw exhaust or irradiated exhaust, these rats receiving the exhaust atmospheres had higher lung dry weight, and they had depressed lung lipid concentration, which was significant on a lung dry weight basis but not on a wet weight basis. As a result, we assumed this reduction of concentration was not due to an edema, but must be due to the dilution of the lipid material by some abnormal nonfunctional lung material. We would like to follow this up and make it a study also.

Since I think I have a few minutes left, I might show you since the close of this study I have just described to you, we have greatly modified our facilities. We have discarded all of the small chambers that you previously saw, for which I am very happy, and have installed 28 of the Laskin-type chambers which you see (figure 13). The chambers are about 44 cubic feet of animal space, not including the pyramid, so they are vertical, flow-type chambers. We are presently doing a great deal of chemistry on them to characterize them chemically in terms of losses. We get much less loss of oxidants and much less loss of NO_2 in these chambers. We get good aerosol distribution. Each chamber is equipped with flow meters, vacuum and pressure gauges, etc.

Our new experimental approach centered around an incomplete factorial in which the numbers in the blocks referred to the numbers of those chambers that will be assigned to each treatment atmosphere (table 1). We intend to study, of course, clean air control, having more of those than any other, raw exhaust, irradiated exhaust, SOX which is SO_2 in the presence of sulphuric acid aerosol, SOX atmosphere superimposed on raw irradiated exhaust, and two NOX atmospheres in which NO will be in ratio to NO_2 in terms of raw irradiated exhaust. This is an attempt to pull out its single effect from the total mixture picture.

The tentative concentrations that we'll have in these atmospheres, for instance, raw exhaust, will run approximately 60 to 80 ppm of carbon monoxide, 25 to 30 of hydrocarbon, we're expressing it now as "methane", 1.5 NO, a trace of NO_2 , etc. (table 2).

The irradiated will be essentially the same except that with the irradiation, the NO of course will be depressed to essentially 0.2 ppm where the NO_2 which initially appears as a trace will increase and attain approximately 1.5 ppm. There will be about 0.3 ppm ozone at the animal's breathing level and

there will be some PAN. Whether the 0.2 ppm is true or not at the present time, I don't know. SOX will involve 0.5 ppm SO₂ in the presence of 100 µg/M³ of sulfuric acid aerosol. Mean particle size runs about 0.5 micron. The NO low/NO₂ high will simulate irradiated, NO high/NO₂ low will simulate raw.

Presently it looks as if our animal loading per chamber will be approximately as indicated in table 3. We should have approximately six activity cages in these chambers also. Our total animal complement for the upcoming study will be somewhere in the neighborhood of 520 animals.

The number of air changes, again, will be 15 air changes per hour.

So, with the new study we intend to follow up the results that we obtained from the past study and, in addition, include dogs for perhaps a closer approach to man himself.

TABLE 1

Experimental Design for Auto

Exhaust - SO_x - NO_x Chronic Study

	CA	R	I
CA	5	3	3
SO_x	3	3	3
$\text{NO}_{1\cdot}/\text{NO}_{2\text{h}}$	3		
$\text{NO}_h/\text{NO}_{21\cdot}$	3		

TABLE 2

Contaminant Concentrations
for Experimental Atmospheres

Poll. (ppm)	(R) Raw	(I) Irr.	SO _x	NO ₁ / NO _{2h}	NO _h / NO ₂₁ .
CO	60-80	60-80	---	---	---
HC	25-30	25-30	---	---	---
NO	1.5	0.2-1.5	---	(I)	(R)
NO ₂	T	T-1.5	---	(I)	(R)
O ₃	---	0.3	---	---	---
PAN	---	0.2	---	---	---
SO ₂	---	---	0.5	---	---
μg/M ³					
H ₂ SO ₄	---	---	100	---	---

TABLE 3

Experimental Animals

Treatments = 8

	<u>Cont. Atmos. (7)</u>	<u>Clean Air</u>
Dogs, ♀ (104)	12	20
Guinea Pigs, ♂ - ♀ (208)	24	40
Rats, ♂ - ♀ (208) <u>520</u>	24	40

Exposure chamber/contaminated atmosphere = 3

Exposure chamber/clean air atmosphere = 5

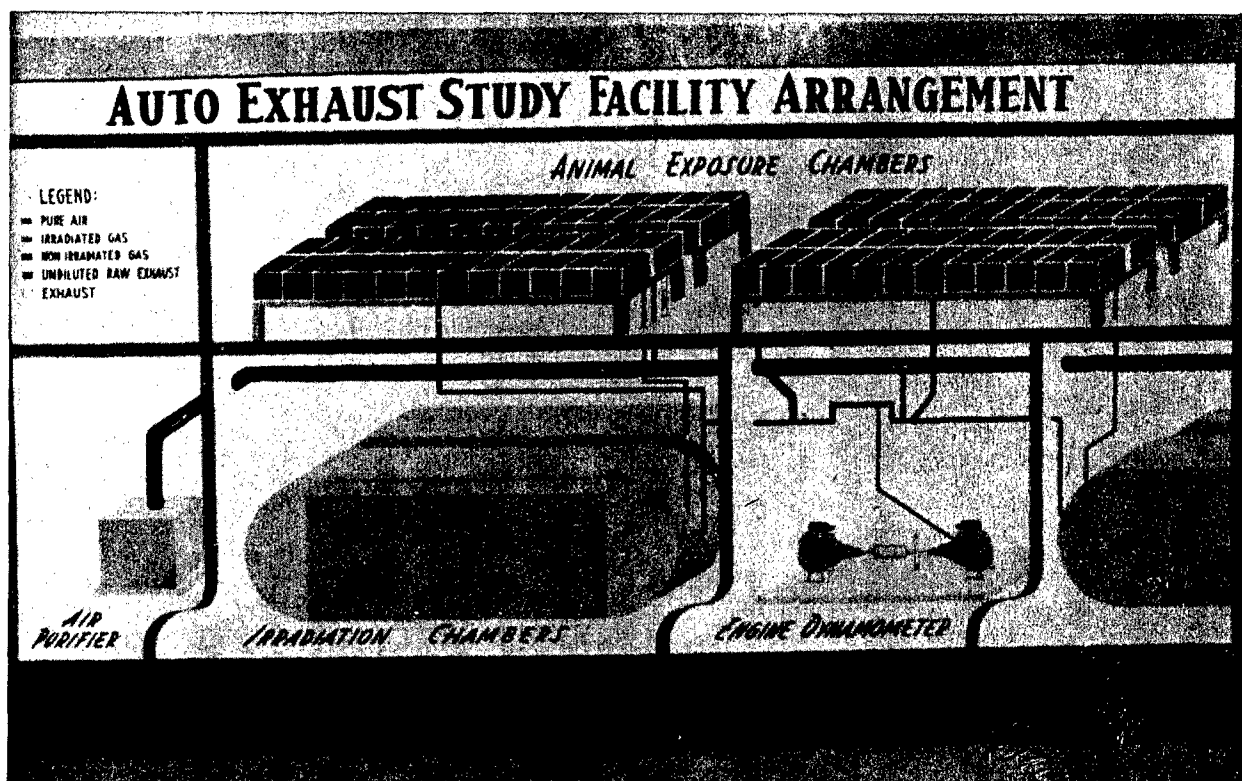


FIGURE 1. AUTO EXHAUST STUDY FACILITY ARRANGEMENT

*PEAK AND TROUGH CONCENTRATION (in PPM) OF IRRADIATED AUTO EXHAUST
CONTAMINANTS OF THE EXPOSURE ATMOSPHERES*

EXPOSURE TABLE NO.	CONTAMINANT				
	CO	NO	NO ₂	HC	O ₃
1	20	0.5	0.4	2.0	0.35
	5	0.08	0.2	0.6	0.1
2	50	1.4	1.3	4.0	0.5
	12	0.05	0.2	0.8	0.2
3	60	1.6	1.55	6.0	0.6
	15	0.05	0.25	1.7	0.3
4	100	2.5	1.6	9.0	1.0
	25	0.4	0.3	2.0	0.5

FIGURE 2. PEAK AND TROUGH CONCENTRATION (in PPM) OF IRRADIATED AUTO EXHAUST CONTAMINANTS OF THE EXPOSURE ATMOSPHERES

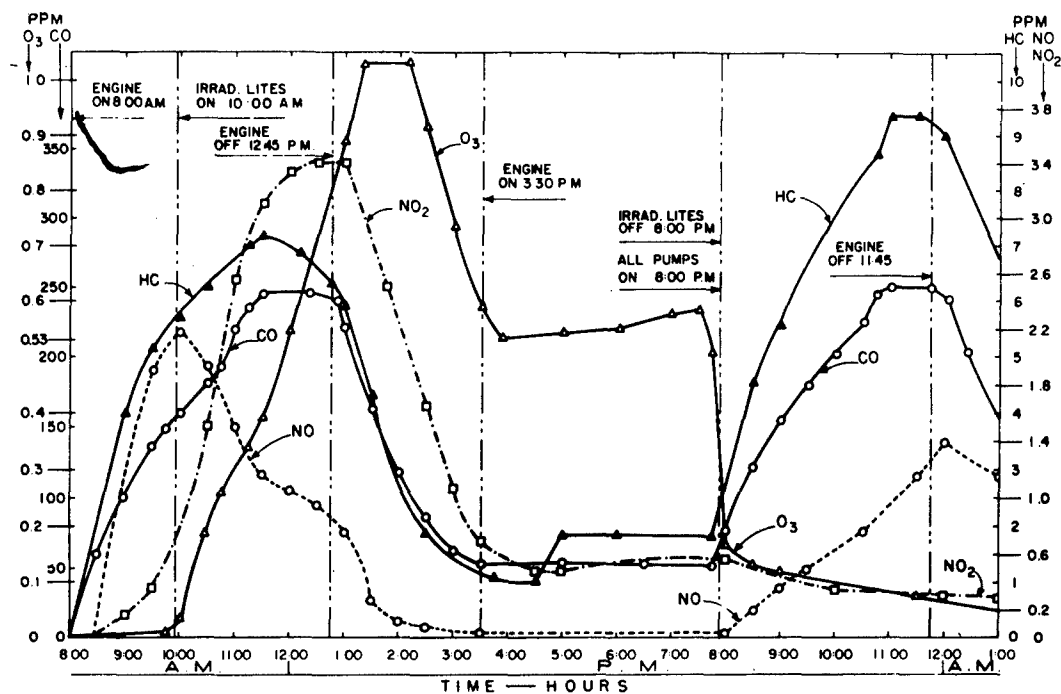


FIGURE 3. DIURNAL VARIATIONS OF IRRADIATED EXHAUST CONCENTRATION - 10 TIMES LOS ANGELES AMBIENT
(Information for these curves from chart data of 1/30/62)

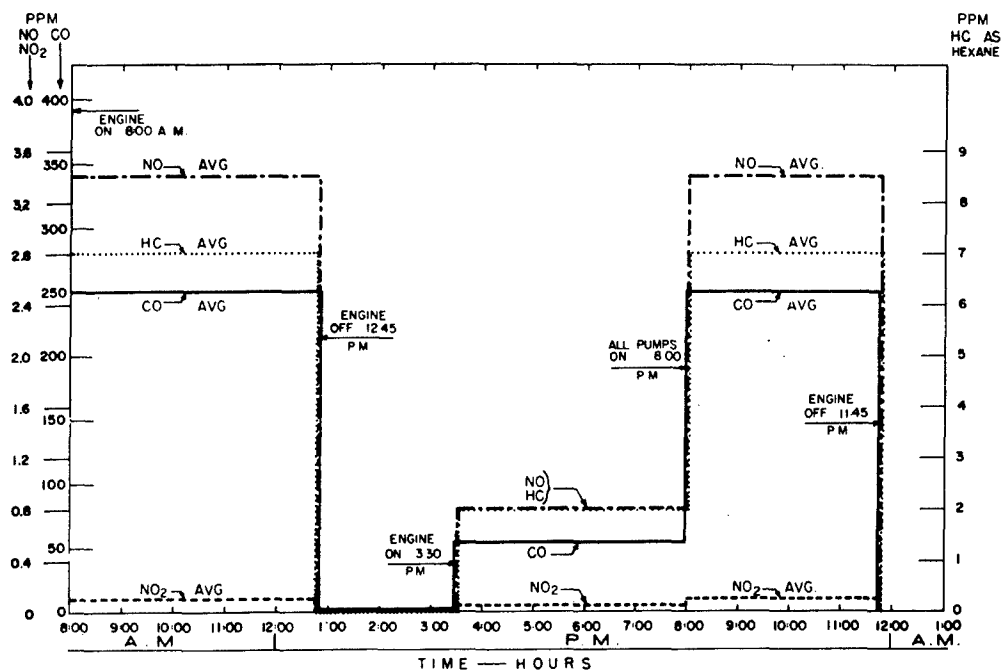


FIGURE 4. NONIRRADIATED EXHAUST DIURNAL VARIATIONS OF CONCENTRATION - 10 TIMES LOS ANGELES AMBIENT

A	A	A	I	O	P
2		2	2R	IGP	L L
GP		GP	IGP	IGP	2R
4H		4H	I	O	IGP
L L		L L	L L	L L	L L
2R		2R	2R	IGP	L L
IGP		IGP	IGP	IGP	L L
L L		L L	L L	L L	L L

FIGURE 5. AN INDIVIDUAL TREATMENT DESIGN WITH BLOCKING EFFECTS

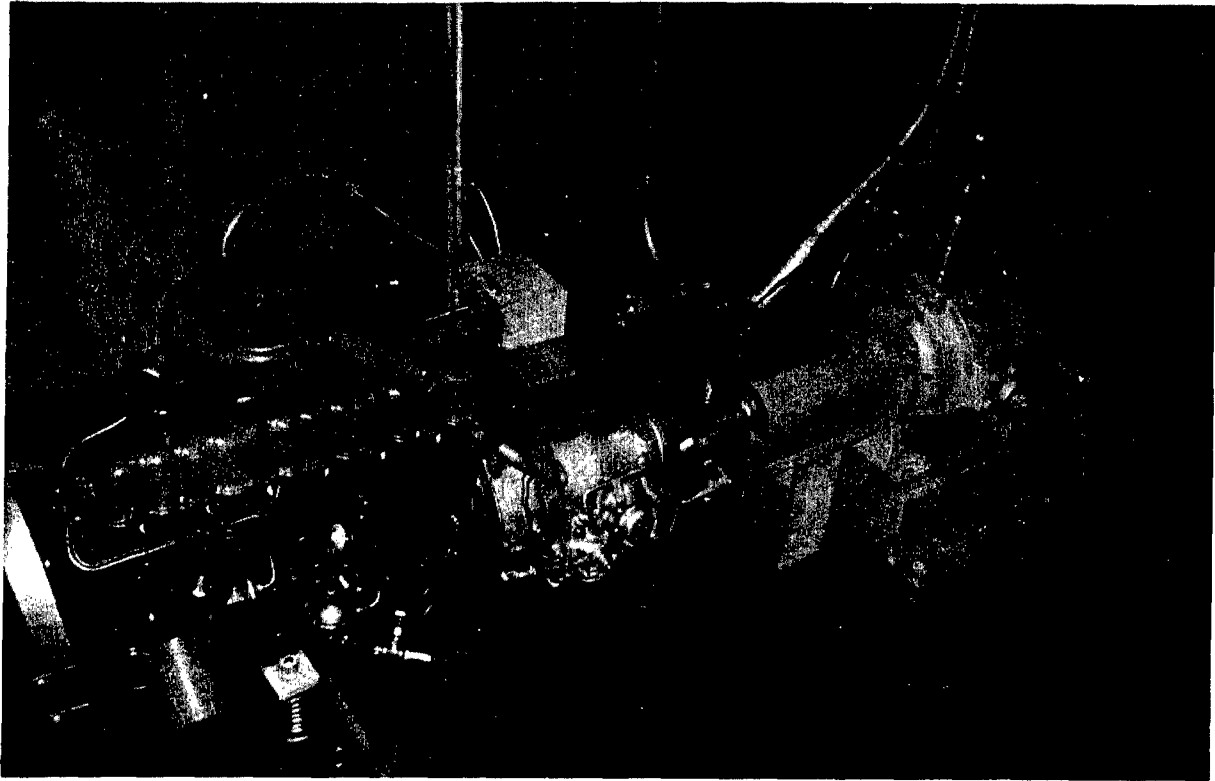


FIGURE 6. STANDARD 6-CYLINDER AUTO ENGINE

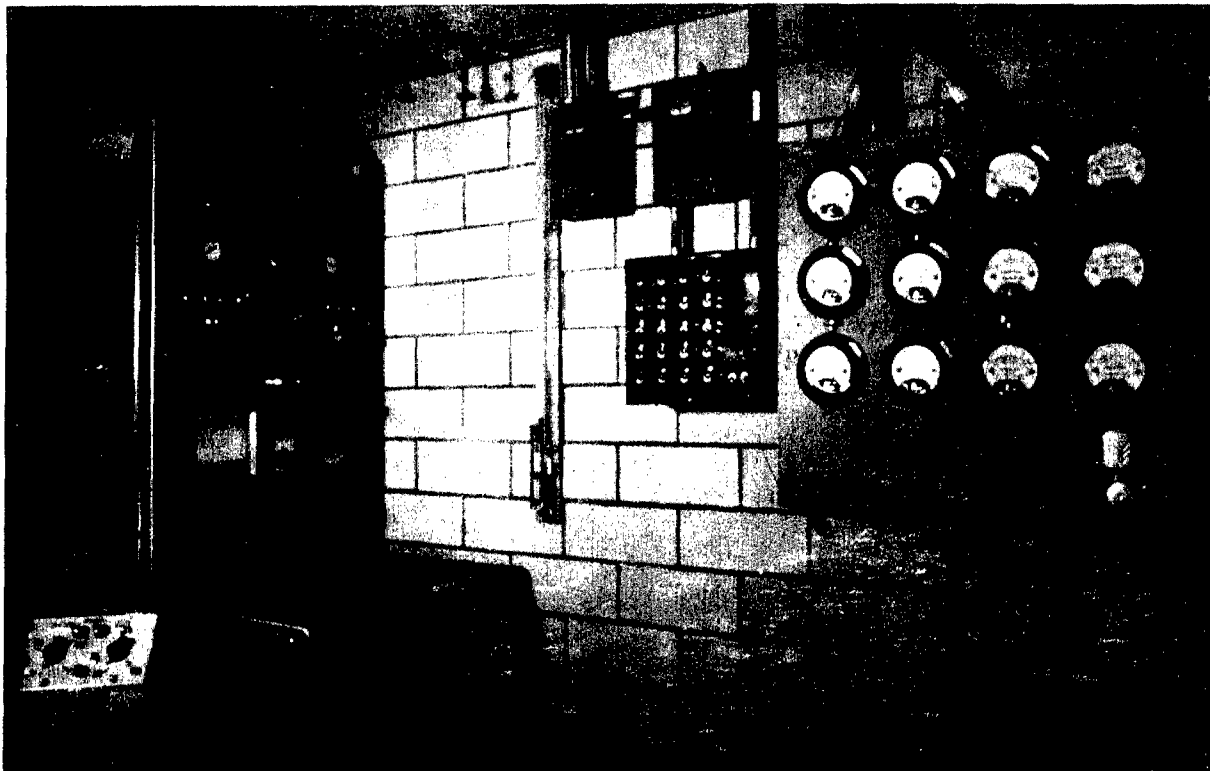


FIGURE 7. AUTOMATIC CONTROLS FOR CYCLING OF AUTO ENGINE



FIGURE 8. IRRADIATION CHAMBER



FIGURE 9. AUTOMATIC INSTRUMENTATION - NO - NO₂ ANALYZER

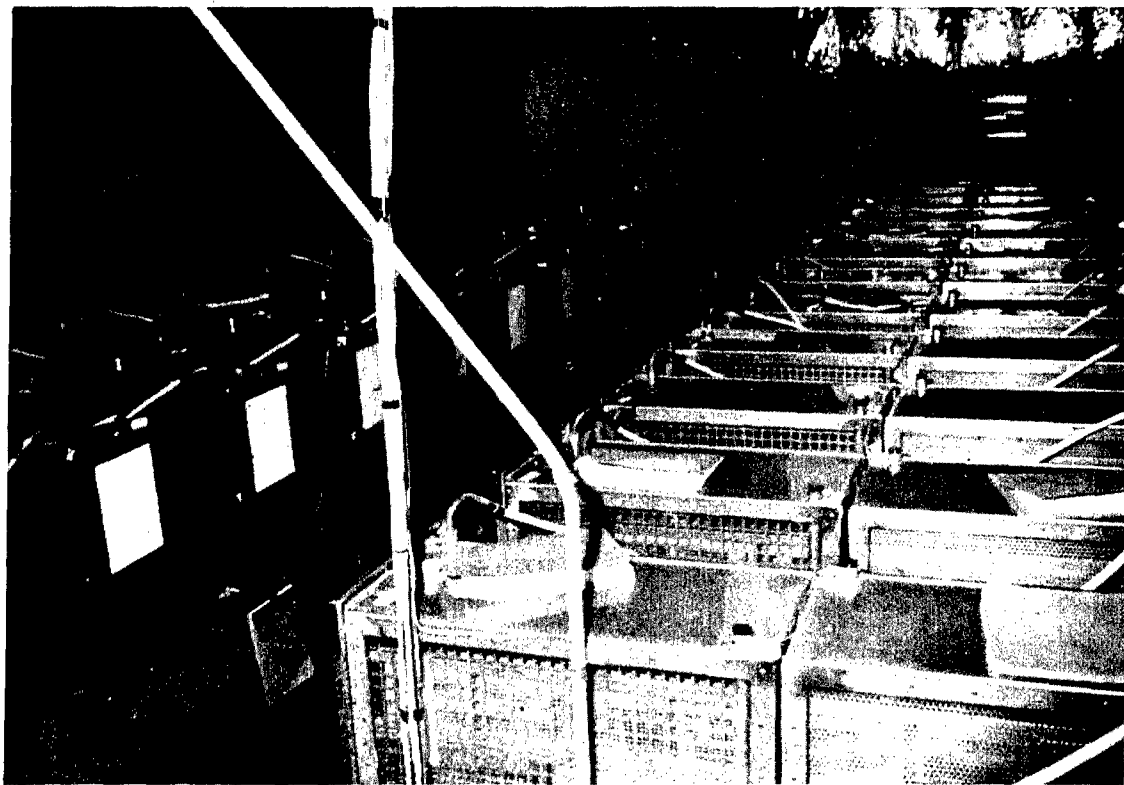


FIGURE 10. HALF OF ANIMAL EXPOSURE CHAMBER ROOM

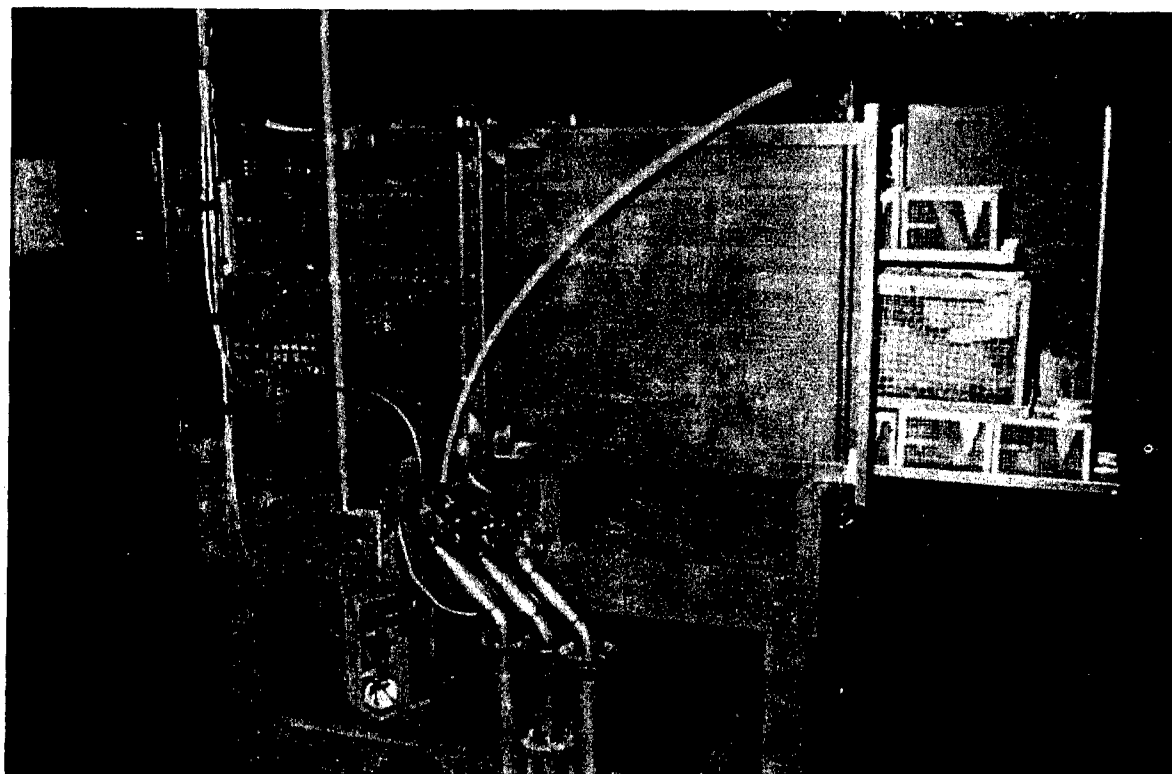


FIGURE 11. TYPICAL CAGE LOADING ARRANGEMENT

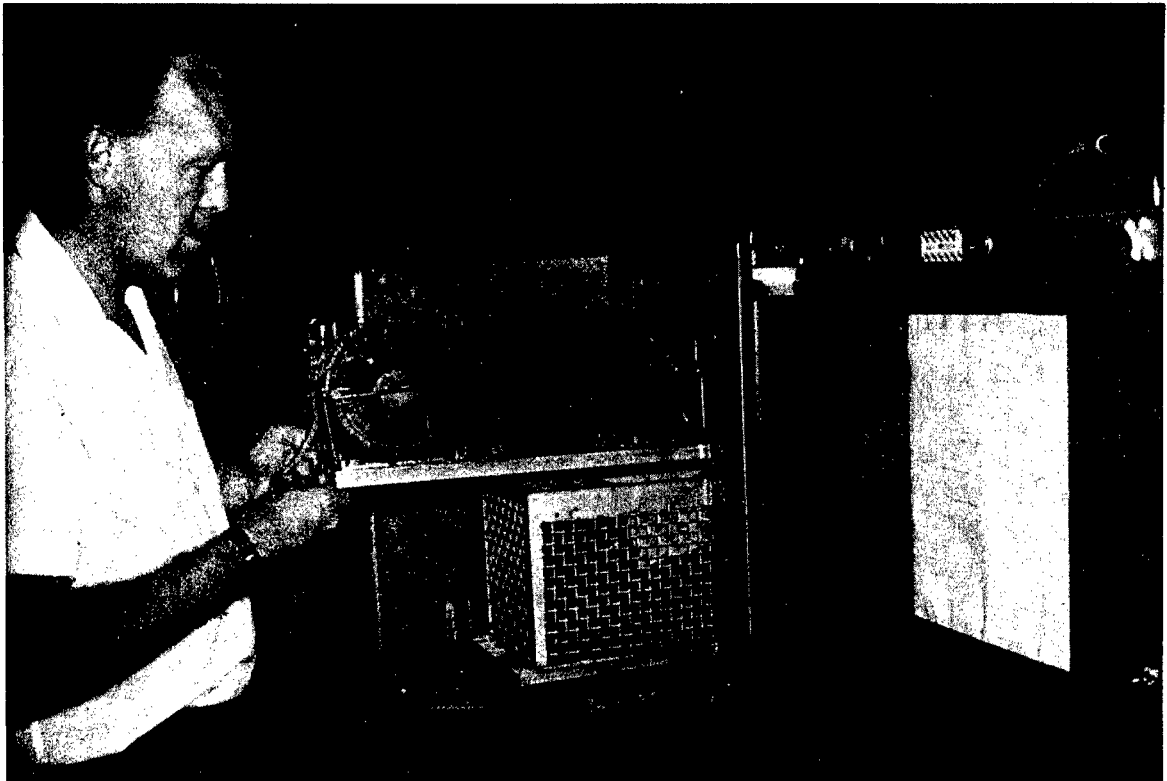


FIGURE 12. SPONTANEOUS ACTIVITY CAGES USED

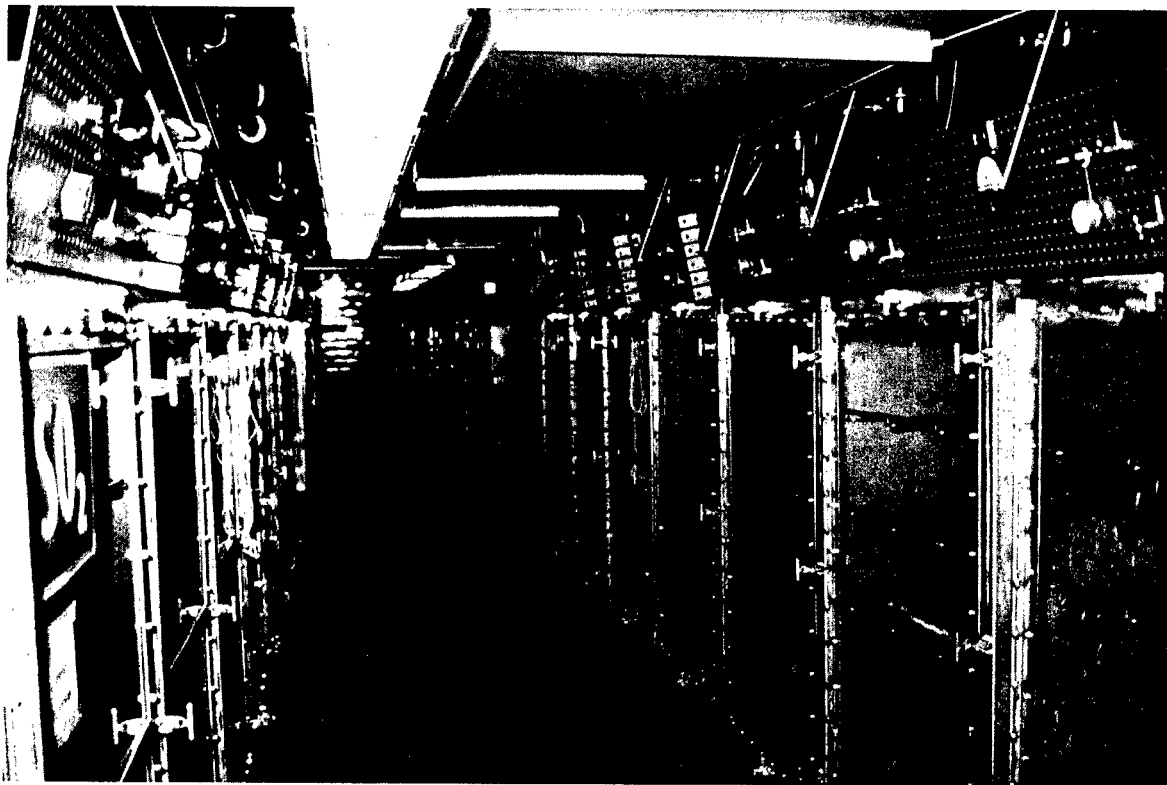


FIGURE 13. LASKIN-TYPE CHAMBERS

PROBLEMS IN THE INTERPRETATION AND EXTRAPOLATION OF ANIMAL DATA TO MAN

By

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In preparing a presentation of this sort it seems to me that there are really three papers one has to write, the one that you prepare originally, the one you give and the one you wish you had delivered when you sat down.

The subject which I have been asked to comment on is one on which, I think, can be found no general agreement. There are some who feel that the risks involved in attempting to extrapolate animal data to man are too great and that man himself must become the experimental animal.

In order to set the stage for this discussion, I think a brief review of the evolution of animal experimentation and predictability of toxicity in man lends some justification for the pessimism that has prevailed on the value of animal studies.

In the beginning, it was customary to use a rat or two, an odd rabbit, and a few mice. Before long it was clear that toxicity in man could not be readily predicted in this way. So, the number of rats increased, and before long someone started statistics, so the number of rats increased still further. Dogs came in. Rabbits went out. Cats became scarce. Now this latter is no joke because during the war, back in the early 40's, I used to meet a not-too-pleasant individual under the Brooklyn Bridge at midnight where I exchanged money for cats. (Laughter)

Well, predictions improved but still there was a long way to go. So the number of rats increased, so did the dogs. So did the mice. More species were added -- monkeys, chimps, marmosets, quail, frogs and pigs. Longer tests were required, ten days, two weeks, six months, two years, to one life span. Still no closer to predictability in man. Once it was just toxicity, and then it was multigeneration tests, carcinogens came in, then co-carcinogens, and if you couldn't find a carcinogen, then you looked for a mutagen. If you couldn't find a mutagen, then you looked for a teratogen. We used not one species but many species. Not one strain, but many strains. Outbred. Inbred. Brother-sister mated. Random mated. Still no better predictability. Once you counted just the dead. This procedure was charged with fallacy, so everything that could be weighed was weighed, and everything that could be removed was sliced and examined histologically. The

function of every organ was looked into. From the cellular, we went to the sub-cellular. Radio isotopes became a must. Physiology gave way to psychology. (Laughter) And now not even the rat doubts the results.

The answer, of course, is trial by ordeal in men, but one might ask how fast do we have to run in order to remain in the same place?

Well, the present state of affairs is really no better, but let's look again at some of the problems that make it difficult to interpret and extrapolate animal data to man.

I think you'll all agree that species and strain differences may alter the response in many cases. For example, rats and mice are known to be resistant to phenols and the aromatic amines. Chickens are highly sensitive to triarylphosphates. The cat is more sensitive to methemoglobin forming compounds than is the rat. There is a fivefold variation in the acute toxicity of sodium iodide in 14 strains of mice. In the recent study on some 16 anti-cholinesterase compounds, weanling rats were shown to be twice as sensitive as adults to malathion and five times as sensitive to Insecticide-7. On the other hand, adult rats were five times more sensitive than weanlings to ONPA. The reason for these differences is not clear but it has been suggested that it may be due to the differences in the activity of the metabolizing enzymes.

The environmental temperature has also been reported to alter the response of chemicals and drugs in animals. With increased temperature, increased toxicity. It has been shown by a number of investigators, some of them are here today, Dr. Diechman, Dr. Keplinger, Dr. Fitzhugh, Dr. K. K. Chen and others.

It has now been demonstrated that animals isolated for long periods of time become different physiological entities. Weanling rats of both sexes, isolated in individual cages, show signs of stress in four to six weeks, and by three months become nervous, aggressive, and intractable. For example, there is an eightfold increase in the toxicity of isoprel by the eighth week of isolation, and a sixteenfold increase by the end of three months. When the significance of these findings is better understood, it may be necessary to modify our present procedure in chronic toxicity and nutrition studies.

Now, one of the most difficult problems in extrapolating the results obtained in animals to man is the species variation in the biotransformation of chemicals entering the circulation. A chemical may be esterified in the rat, conjugated in the dog, acetylated in man. The difference in response may be qualitative, as in the case of morphine, which is a CNS depressant in the dog, stimulant in the mouse. The biochemical transformation may be also quantitative as in the case of butylated hydroxyanisole.

Now, note in this study by Estes and coworkers at the University of Rochester, really at Eastman-Kodak, that the rabbit and the rat excrete the BHA 60 to 70% as a glucuronide, whereas the dog excretes only 5.5%, and 56% as unabsorbed material.

In the case of man, from this dose of 0.4 milligrams per kilogram, most of the material is excreted as a glucuronide. Now the point I want to make is, had you used the dog as the sole experimental animal, one may have come to an erroneous conclusion. But the high level of nonabsorption could have caused the speculation about the complete nonabsorption of the material from very low doses, since this material was to be used as a food additive. So it's essential that metabolic studies, biotransformation studies, be conducted in a number of species. Now, not only may the route be different, but also the rate at which the reaction occurs. A single dose of meperidine is metabolized at the rate of 20% per hour in man and 90% per hour in the dog. Had one used the dog as the sole experimental animal, one probably would have discarded meperidine. The type of response depends considerably upon the relative absorption, and the site of maximum concentration may or may not be in the tissues upon which the chemicals exert their characteristic effects.

For example, digitalis has its highest concentration in the liver but its primary site of action is on the myocardium.

Excretion rates will vary widely but it is obvious that the more rapid the excretion, the less likely are the chances of accumulation and undesirable effects. Rapid excretion, however, may also cause kidney injury. It has been pointed out that almost all drug effects seen in man can be demonstrated to have a counterpart in animals, but it must be recognized that drug effects seen in animals cannot readily be translated into their counterpart in man. For example, only a few of the anti-tumor compounds that have been found to be effective in animals have any effect in man.

Bis-dehydrodoisynolic acid, synthesized some years ago, was a potent estrogenic compound in animals, but when tried in man had essentially no effect.

A very interesting paper which appeared only a few weeks ago in the Journal of the American Medical Association was concerned with nonspecific ulcerations of the small intestine as a result of patients being treated with hydrochlorothiazide and taking enteric-coated potassium chloride. Now a retrospective study indicated that there is a fairly low incidence of this nonspecific ulceration but when all of the cases had been reviewed, it was quite apparent that it was not due to the compound itself but rather implicated potassium chloride. Now, this was an effect that had gone unseen in all the experimental work that had been done over the years with potassium chloride. When the investigators went back and did studies in monkeys and dogs with enteric-coated potassium chloride, they found that in the monkeys this effect could be

seen very easily, but to a much lesser degree in the dog. So here is an effect when it is seen in man that has gone unnoticed until animal experimentation.

The principles outlined above are only a few of the facts that might affect the responding system when drugs and chemicals are administered by any route. We've heard during the day from Dr. Innes, Colonel Reeves and Dr. Spencer about the many other factors that would enter into interpretation and the extrapolation of data from animal experiments to man.

Now since this Conference is primarily concerned with atmospheric contamination in confined spaces, I would like to point out some of the variables in relation to studies that make interpretations very difficult. It has been our experience, as well as that of others, that nominal concentrations for inhalation studies are of very little value when attempting to extrapolate animal data to man. We see this so much in having to search the literature only to find these kinds of data. The chemical reactivity and the adsorption on the walls of the chamber may markedly alter the concentration and the chemical being introduced. A prime example is nitrogen trifluoride which is rapidly adsorbed on the walls of the vessel so that analytical determinations of concentrations to which animals are being exposed is an absolute necessity if one is to make a proper evaluation of the physiological and toxicological effects.

Extremely low humidity can dry the nasal passages of exposed animals and render them more susceptible to irritating gases. On the other hand, high humidity places an additional physiological stress on the animal and may also alter the reactive gases such as fluorine and oxygen difluoride.

Last week I had a very rare privilege of visiting one of the most distinguished authorities in the field of thermodynamics and kinetics. I was very much concerned about the remarks that one hears in the discussion concerning these very reactive gases, fluorine, oxygen type fluoride, etc., and I thought maybe it was time that someone started the ball rolling to see if we couldn't get some idea of the thermodynamics of these materials. Dr. Beckett is a world authority, and he gave me an hour lecture on the materials with which we are all very much concerned. I can only say that this is one area that we're going to have to look into very carefully, that we can no longer simply introduce a gas into a chamber and simply assume that this is what we are measuring, or that this is the effect we are getting due to this. We know the thermodynamic reactions of fluorine to yield HF. What we don't know, of course, is the kinetics, and much to my disappointment, there is very little known about the kinetics of these exotic materials, but it is one thing that I'm quite convinced that we are going to have to look at much more carefully.

For example, in the case of FLOX and hydrazine thermodynamically, the question now is that you'll get (and it has already been identified) such things as CF_4 and/or carbonyl fluoride, and these carbonyl compounds have

always presented a real problem to us. They are going to continue to present problems and we'd better be prepared to do some additional work.

Now, one is always concerned about the possible ingestion of significant amounts of material from animals licking at fur or eating contaminated food, and yet I can find nowhere in the literature where this problem has been decided definitively or how much of this contamination of fur and food actually contributes to the overall toxicity of the compound.

There are many reports of animals exposed to chemicals under static conditions and this poses an enormous problem of interpretation and extrapolation. This method, however, has severe limitations since the experimental concentration does not remain constant, and the duration of exposure is limited by the decrease in oxygen and the building up of CO₂.

There are, of course, situations where it may necessitate one having to use this type of procedure, particularly where extremely small quantities are available, but it is hoped that more attention will be given to inhalation studies using dynamic flow.

Again, these are only some of the factors. There are many more, I'm sure, that you could think of better than I.

I want to say a few words about safety factors. It has generally been assumed that man is at least ten times as sensitive to a chemical as the most sensitive species studied. Hagan has stated that man is about six times more sensitive to drugs than dogs. Our clinical experience with lethal amphetamine poisoning indicates that the average fatal dose is about 3 to 4 milligrams per kilogram, which is about one-sixth of the oral LD-50 for amphetamine in dogs. Whatever the rationale of safety factors, the simplest method of determining the acceptable daily intake of a chemical or a drug is to divide the no-effect level as determined in the most sensitive species by an arbitrary factor, usually 100. Now, there has been much criticism about this method since it has no real scientific basis. Nevertheless, it has been used extensively and in most instances has, if anything, erred on the side of safety. A more scientific approach has been proposed by taking into account the slope of the log dose response and its standard deviation. The acceptable daily intake could then be estimated by the threshold doses less 6 standard deviations. Well, the chances of this dose causing the effects studied was about one in a thousand million. The major difficulty is the determination of the threshold dose by extrapolation of the log dose response curve. Well, Mantell and Brian attempted to overcome this difficulty by extrapolating to the acceptable dose by means of an arbitrary shallow probit slope of one normal deviate per log. This gives about one in a hundred million. Where the effects are definite and predictable, the extrapolation of animal data to man in this way seems justified, but when the effects are delayed and unpredictable as in the case of tumors, extrapolation of this kind of data in this manner does not seem warranted.

Now, for many years, inhalation studies on many species of laboratory animals have been carried out on the basis of either acute exposures lasting several minutes to several hours or by repeated exposure several hours a day up to 7 hours a day for several days, for 5 days a week, and for several weeks. As a result, a vast amount of information has accumulated and indeed this has provided the basis for the threshold limit values.

With the advent of the nuclear submarine and the spacecraft, there was an immediate need for estimation of what we've called "design criteria" for the construction of the nuclear ships. Our first objective was to attempt to establish levels for 1-hour, 8-hour and 24-hour exposure. At that time we were not thinking of the long duration. You see in this chart the recommendations that have been made by the Committee on Toxicology of the National Research Council for 1-hour, 8-hour, 24-hour exposures to such things as benzene, formaldehyde, mercury, nitrogen dioxide, ozone, toluene and xylene. Now these estimates, and indeed they are only estimates, have been based on the available toxicological data and good judgment on the part of the Committee. Now in many of these one can, of course, use the CT concept in extrapolating for short-term exposure, but here again, this method has its limitations.

It was suggested by some group in another part of the country that perhaps one simply could multiply the threshold limit values by an arbitrary factor, let's say 10, 100 or 1,000, and use these for establishing short-term levels. Well, obviously such a method as this could not be condoned.

Here's a group of materials for short-term exposure. In this first case of carbon monoxide, part of this involves the classified problem, but I think that it will illustrate the problem we have in trying to extrapolate data to man. Now in the case of carbon monoxide, I think we'll all agree that this is a fairly simple method of procedure. We are dealing with material that has merely a physical-chemical reaction, combining with the hemoglobin on a physical-chemical basis, it is taken up easily and given up easily. So I think we can compare quite closely the levels that produce effects in animals, that those levels will also be practically the same in man.

And, if you look at some of the early studies on carbon monoxide, it certainly is very true. Now, we're concerned with how much a man could conceivably be exposed to for a matter of 1 hour, 30 minutes, 15 minutes, of carbon monoxide. These are the levels that we think one can be exposed to without any serious impairment of function, but nevertheless there's another phase known as "mental acuity" and it has been shown that at certain levels mental acuity is very much affected by levels of carbon monoxide and this has been shown by various types of tests -- "t" tests, crossing "t's" and dotting "i's" and so forth.

Now, the question was then, to the military, if this involves a man carrying out a mission, and if mental acuity is affected, then we would have to reduce the levels to 200, 500 and 1,000 for 15 minutes.

I think you can easily see what a problem one has here in the case of fluorine and hydrogen fluoride, oxygen difluoride. The amount of data available in inhalation studies with fluorine has been very limited. The earliest work, I think, being done by Dr. Stokinger at the University of Rochester during the days of the Manhattan Project. There have been a few other studies - oxygen difluoride, a recent study, I think, will be reported down at the meeting of the American Industrial Hygiene Association, but again, only one other paper on oxygen difluoride by LaBell in 1943, I believe about that time, so this is all the data one has. We can't simply sit and wait. We are asked to make some sort of prediction. We need continuous and further short-term exposure studies.

Just a word about UDMH. The Committee on Toxicology of the National Research Council a few years ago set the following levels for UDMH (unsymmetrical dimethylhydrazine) of 10, 20, 35, 50 ppm for 60, 30, 15 and 5 minutes, respectively. About six months or a year ago, the Sub-Committee of the American Industrial Hygiene Association also published what they thought were acceptable levels for short-term exposure and there's a wide divergence of opinion, so that when we're talking about extrapolating data, interpreting data, you must remember that we've got the human factor because we don't all think alike, and we don't all come up with the same answers. Now the difference between theirs and ours is quite great. Their levels are 600, 200, 100 and 50. Now one must also remember that the time these were set we had very little data to go on, and since that time there has been considerable more data published and made available to the Committee. Now, the Committee on Toxicology has, and is now reviewing, the data on UDMH with the hope of revising these levels. I can say, however, I think the levels that will be suggested will be somewhere between 600 and the original 50.

Here's one of the errors that always gives us a great problem of sensitization and tolerance. How do you crank this into an evaluation of extrapolating animal data to man? If the animal does show some degree of sensitization, is this going to carry over to the human? This means more human studies and not extrapolations. You've got two types. You've got the skin sensitization and you've got lung sensitization. One is fairly easy to reproduce in animals. The other is much more difficult. You come to the question of tolerance and here we have three areas -- one acting at the sensory nerve ending; another on the emetic center; another on the myocardium. How do you extrapolate this kind of data and interpret it? If man develops a tolerance for sulphur dioxide, does that mean then that you can keep on raising the level of sulphur dioxide? To what extent can you go?

We know that patients develop a tolerance to morphine of the emetic response but still maintain many of the other effects that are well described.

One never loses the constipating effect from morphine. He doesn't lose the analgesic effect, but he does develop some tolerance.

The interpretation and extrapolation of animal data to man has never been easy and clear-cut and especially where there are no benefits to be derived. By that I mean it's a great deal easier to work on the toxicology of a compound when, let's say, you are developing a therapeutic agent, where you have some end point you always measure. But in the case of so many of our chemicals, we have no such end point.

I started out 30 years ago working on adrenal physiology where everything that was done in every species seemed to have its counterpart in man. The advances that have been made in developing therapeutic agents by use of animal experimentation are certainly evidence that there must be some relationship. The question is how to find it. There are a number of things that need to be done, but there are several that I would like to emphasize.

First, and this has been said over and over again, but I'm going to repeat it also, is the careful selection of species. Not only from the point of view of the pathogens and many other problems that may be inherited, but I think one must stop and almost make a review of the animal kingdom and see wherein this species fit into the kind of a problem we are trying to solve. I remember telling you at Lockheed of the story of the man at the University of Pennsylvania who was trying to prove that the kidney was a secretory organ. I think if he'd left it to some of us we'd probably never have answered it and it would have taken 20 years, but his first approach was to sit down and go through the animal kingdom until he found a species or form that he could use to prove his theory. It turned out to be the goose fish because it had a tubular kidney, and demonstrated for the first time that the kidney was secretory. I think this sort of approach has to be applied to a lot of our work as the kind of animal we're using, the sort of thing that will eventually prove our point, and not just a species to count the dead. We must have more studies in metabolism. From our constant review in our center where we have information on pretty close now to a quarter of a million compounds, we see the paucity of information on metabolism and it makes the whole question of interpretation and extrapolation very difficult. There must be better analytical methods, especially in our inhalation work. We're being satisfied, really, with too crude methods. We must have, in addition, preplanning and regular periods of assessment, and I can't help but recollect in the early days of my research, working with a man who did just that. It was a reassessment, not every month, not every six months or a year, it was every day. A reassessment of what we did yesterday, what we are going to do today, what the data looks like in light of the direction in which we're going. I think this is most important.

Then, we must have, as I've already mentioned, a thorough knowledge of the thermodynamics and the kinetics of these reactive materials.

SESSION III

PANEL ON MINIMUM CRITERIA AND TRADE-OFFS

Chairman -- Dr. H. E. Stokinger

Members -- Dr. Arthur DuBois

Dr. Elliott S. Harris

Dr. Harry W. Hays

Dr. Harold C. Hodge

Lt. Col. Johnie L. Reeves

Maj. Herbert H. Reynolds

Mr. Verald K. Rowe

Capt. Jac Siegel

Dr. A. A. Thomas

DR. KITZES: Good morning, I'm glad to see that many of you have come back because I feel that this morning will be the real critical point in our Conference. The real essence of the Conference will be discussed today. The purpose for getting together this Conference was actually to exchange ideas, not essentially to provide new information. As we noted yesterday, there still remain a tremendous number of problems in the field of toxicology in providing the answers not only for the Department of Defense but for industry and for the basic research that is going on in many universities and institutes in the country. The Conference wasn't called to solve these problems completely because we know they have existed for many, many years.

As pointed out by one of the speakers yesterday, there has been a slow evolution in procedures and approach to obtaining toxicological information; however, today we thought that, rather than continue presenting data and experiences, we would start at this point to have a group of experts get together and try to answer the questions if not already existent in your mind that may have originated from the review of our problems yesterday. We realize that with the working installation of the large facility out at Wright-Patterson, with the need for data that has to be applied throughout future aerospace systems, we cannot wait to indulge in academic philosophies or discussions. We need answers that can be applied tomorrow in the solution of problems in the prediction of men in our space systems; therefore, we will have a panel today that will try to set the minimum criteria, keeping in mind the course involved and the tradeoffs. Tradeoffs mean that we would like to obtain the maximum benefits from many of the works that we can initiate and perform with our facility at the Base. Tradeoffs, again, in terms of not only data -- data that is applied to animals -- but the problems in terms of extrapolations of data from animals to humans; we are primarily concerned with the human subject. Any work, anything that can be done with animals or possibly with human subjects, and then can be applied directly to the protection of the man system, would be of tremendous value to us; and so, with Dr. Stokinger as Chairman of this panel, we would try to provide the guidance that is necessary to set up the minimum criteria and tradeoffs. Dr. Stokinger, again, is well known to many of you. He is the Chief Toxicologist of the U. S. Public Health Service, Division of Occupational Health, in Cincinnati, Ohio. He is a graduate of Harvard and Columbia and has spent many years in the area of toxicology at the University of Rochester. Dr. Stokinger --

DR. STOKINGER: You've heard that fine introduction that outlines the purpose of the panel this morning, and I think you are all familiar with the panelists, with the exception of a few -- that is Dr. Arthur DuBois. He is a Respiratory Physiologist with the University of Pennsylvania in Philadelphia. The other member that may not be familiar is Dr. Harris. He is at NASA. Then we have our panelists together -- we are ready. As in the start of a tennis match, the players are ready. I personally checked each

panelist this morning and found that all the bodies are warm, they are breathing and their pupils are reacting to light; which, of course, is one instance of minimal criteria, one of the subjects for discussion this morning.

Now I think the panel will try to organize some of the material that was delivered yesterday, and we will start out with Topic Number 1. What are the minimal criteria that must be met in continuous exposure work to assure comparable data reproduced by various investigators? What are the conditions that lend themselves most effectively to standardization? How much deviation is acceptable? And Part B of this topic is, what are the minimal requirements for the production of biologic data; frequency of biologic samplings, including pre-exposure baselines; biologic statistical methodology; and the maintenance of animals during exposure, to allow direct comparisons of the findings. Now I think I will start the ball rolling with a contribution from Panel Member Siegel, and I think he has a table to fortify the discussion. Capt. Siegel --

CAPT. SIEGEL: Before I start, last night about 15 of us went to a real swanky restaurant, and we are not allowed to mention the name because we don't accept unpaid commercials on this station here, but what do you think the specialty of the house was? -- Rabbit. (Laughter)

About two years ago, we had a meeting similar to this at Lockheed, and at that time we suggested minimum criteria that might be standardized. I would like to discuss these minimum criteria, or at least the basic things that we think should be standardized. All these things were discussed yesterday, but I think it is worthwhile taking a few minutes and going over them. They are set forth in this table.

TEST CRITERIA WHICH MIGHT BE STANDARDIZED

- Number and species of animals
- Air changes per minute
- Temperature and humidity
- Particle size (mist and dusts)
- Length of exposure (90 days)
- Methods of analysis
- Minimum signs to look for
- Minimum blood work
- Minimum pathology
- Biochemistry (?)

I'm going to comment on each of these items in relation to what we have done.

Number and species of animals -- We have used five species of animals -- rats, rabbits, dogs, guinea pigs and monkeys. In numbers, we used 15 rats, 15 guinea pigs, 3 rabbits, 2 dogs, 3 monkeys. The reason I am mentioning this, we need some basis for comparison, in comparison to other laboratories.

Air changes per minute -- We have two air changes per minute in a chamber that is two cubic meters in size.

The temperature is kept between 75 and 80 degrees, and the humidity is kept close to 50 per cent.

Particle size -- We have not run any dusts up to this point. All of our experience is with aerosols. The maximum particle size is five microns, with a mean mass diameter of two and one-half microns. We don't know how low the particles go. So it would be a spectrum from something probably below a half a micron to about two and one-half microns.

Length of exposure -- We have run continuously for 90 days and longer, but we are making our comparisons on a 90-day basis and, in addition, we are running these 30 repeated 8-hour exposures, 5 days a week, on every material we study so that we might have a comparison later on and perhaps cut down on these 90-day exposures if there is any validity to a comparison.

The only thing I can say about methods of analysis is that we do not rely on a single method of analysis for any material. We will do wet chemistry, we will do instrumentation and we will do gravimetric analysis if appropriate. We will use at least two methods, because I think it is the key in running inhalation studies. As was mentioned yesterday, unless you know your concentrations, not nominal but the actual concentrations, you really don't have an experiment. There has been a lot of stuff in the literature that is really -- well, we would be hard pressed to interpret, because we don't know what the concentration is.

Minimal signs to look for -- Well, we've had some discussion here yesterday that you look for everything and I think we ought to arrive at some position. Do we want to look for everything, or do we want to at least look for the minimal amount of things and leave the rest up to the different laboratories depending upon whether they are doing some real basic work or some screening work? It is a judgment that depends on the objective of the work. I am sure that the more that you can see, fine, but the less handling that you can do, you are better off too, particularly on a continuous exposure. Again, in relation to minimum of blood work, we have decided that we will not remove the animals for blood work during the course of the run. We will do it before. We will do it after. But we will not stress the animal during the run.

Minimum pathology -- We've done an awful lot of pathology with very, very little positive contributions to the picture, and we don't know just where to start or stop. But we do know that if we don't do pathology, we are going to be criticized. This is one of the reasons we are doing pathology.

Biochemistry -- Well, we have had many things outlined -- what do we do in biochemistry? I am sure for each material you've got to look for a

different part of the cycle and pick out a particular material that might have promise based on the literature, on known mechanism or on what is known about the decomposition of the product. But you cannot do everything. You are limited by manpower, by space and by time. I might add one more thing to this list, which is not on the table, and that is the possibility of X-raying the lungs of all the animals, even the small animals. We think that this might be a useful tool. I would like to stop at this moment and let others continue the discussion.

DR. STOKINGER: Thank you. You've heard Dr. Siegel, among other points, come out in defense of only occasionally handling his animals. On the other hand, you heard yesterday morning Col. Reeves present a diametrically opposed view of frequent, if not daily, handling of the animals, to obtain a record of these progressive changes that may be occurring. Col. Reeves, would you like to say anything in defense of this position so that we could come to some uniform agreement as to which is the better procedure? Is stressing important here? Could it be subjugated to the benefits derived from frequent samplings, getting all this information? This is a question of fundamental significance because if you alter your animals by taking frequent blood samples, it stresses them and there may be an altered response due to the handling and not to the toxic agent. Possibly Dr. Reynolds might have something to say about this from the psychological standpoint too. Would you like to address yourself to this?

LT. COL. REEVES: Well, insofar as examinations are concerned, the physical examinations, manipulative in nature, I should think that the more frequent observation you can make the better off you will be. I can't really understand a position which relies on essentially a "live or die" kind of toxicology; or that relies on an approach in which an animal is examined before anything happens to it and then allows a long time to pass between that observation and a follow-up observation some 90 days later. This doesn't make good sense to me from the point of view of being able to follow the animal's clinical progress. Now then, insofar as frequent blood sampling is concerned, I will certainly concur with the comment that you can't take daily blood samples. An inventory of my list of suggested minimums indicates that they will require about ten and a quarter milliliters of blood assuming a normal hematocrit. We could probably reduce this somewhat by going to, or developing, ultramicro methods. However, with the methods available to us now, it is going to take around ten milliliters of blood. To withdraw this quantity of blood from an animal such as a dog or a monkey any more frequently than once a week or so is certainly going to stress it.

DR. STOKINGER: Dr. Reynolds, do you have anything that you might say about the handling, fondling or disturbance of animals in regard to frequency?

MAJ. REYNOLDS: I can only say what we find in the literature, and that is that there is no doubt but what animals that are handled more frequently during experimentation provide different results than animals that are handled less frequently. Krech and Rosensweig, a group at the University of California, has shown that there is change in the cholinesterase in the brain, particularly at the subcortical level, as a result of handling and enriched environments; and so handling does produce a different animal, one that is less reactive to stress because he has been handled and one which will in time show less variability in experimentation. Thus, I think that this is something that must definitely be considered in terms of working with animals from day to day. If you handle all of your subjects alike, then supposedly handling would not produce any significant difference, but if you handle some more than others, then this becomes a very difficult thing to quantify. So this is the thing I think an experimenter must consider.

DR. STOKINGER: Thank you. You've heard Col. Reeves put this matter of frequency sampling on a rather quantitative basis. But you've also heard from Dr. Reynolds, that it's a little more difficult because of the psychologic aspects to quantify this. Could you make some sort of an attempt to say how frequently you can handle the animal and yet not produce a stressful situation that would interfere with the measurements you are trying to take. Can this be put on a roughly quantitative basis, say once a week, once every two weeks, or how do you handle them? Can you put it in similar terms as the former speaker here?

MAJ. REYNOLDS: Well, I think it would be very difficult for me to be this exacting in reply. Some investigators have developed automatic types of devices that handle the animal in various types of psychological experiments. For example, to see that they do not touch the animals themselves, but that the animal is mechanically picked up and placed in the experimental situation, or is released by various mechanisms into the experimental device and so on, in order that they will not be handled. I think that there are many potentially relevant variables here since we know that if you handle an animal at a given age it is different. In other words, if it is a young animal and you handle it a lot, this is different from handling it when it is an older animal. Animals that are handled just following weaning, for example, or during the first few days of life, present different responses than animals that may be handled somewhat later on in life. So I think it is an extremely difficult thing to decide upon. I think that what Dr. Reeves is suggesting is that we need to know a great deal about the physical state of the animal, and to make sure that the animal is a healthy and qualified experimental animal. I am sure that there is a good deal that we can all agree upon to make sure that we are working with an animal that will provide valid results from the standpoint of his physiology.

DR. STOKINGER: Here I think we have one of the situations where controls do not serve any useful purpose, because if you upset some factor in an animal and then superimpose a toxic agent that gives a similar response,

this, of course, cannot occur in the control which has no original response to be potentiated. So controls cannot serve any useful purpose in this type of situation.

DR. HODGE: I am just wondering. I remember hearing Dr. Hays yesterday afternoon mention an increase, I think it was 16 times, in the toxicity of amphetamine if an animal has been kept for prolonged periods in solitude. If we are going to send our spacemen off for weeks and weeks, all by themselves, shouldn't our experimental animals be placed in solitude so that we set up an animal model that includes this stress?

DR. STOKINGER: Does anybody on the panel want to answer this?

DR. THOMAS: Well, you recall the old joke about the first couple of astronauts. They asked one of them: "What do you plan to do up there?" He said: "I'm planning to cry a lot." He was pretty much alone, especially in the early systems. But I don't think that if we get into this area of combined stress we should start simulating other things, like weightlessness and so on. This just is not feasible. We have enough problems in just trying to bring toxicology to a common basis here. The other thing that I would like to bring up is the matter of economy. If we handle animals and get data, any kind of data, whether it is a good physical examination which points out the first neurological deficit, or a clinical laboratory test which becomes abnormal, if I can cut off a 90-day exposure after the second week, I would save a lot of money. If I wait until the end to get my samples, then yes, I might find that I have gross changes in biochemistry and other things, but we are all being pushed for time, and so I think that there might be a tradeoff here. What do you think about this?

DR. STOKINGER: Does anybody want to answer that?

LT. COL. REEVES: I think this is, in essence, what I have said.

DR. STOKINGER: Are there any further comments on this "tradeoff" question? I think we are getting a little ahead of ourselves, but these two topics, minimum data and tradeoff, are sort of interrelated and hard to separate.

DR. HARRIS: I have something to say on that. First of all, on the question of solitude, we have just seen the Gemini flight, in which there were two men covering one another; also, there is the question of how you define solitude. These men are in continuous contact with each other and earth stations. I am very much in agreement with Dr. Thomas with respect to the matter of cost. If you run a 90-day run in 5 PSI oxygen you have an expensive proposition. If you can cut it off in two weeks, or three weeks, you are a lot better off. Combined stress runs into a much more complex problem.

DR. STOKINGER: I think we will conclude this aspect of stress to responses. I would like to address a question to Dr. DuBois along the line of the use of respiratory function tests in animals. Is this a useful criteria? I'm surprised that it was not highlighted in greater detail than it was yesterday. It was only referred to, as I remember, by Dr. Hueter from SEC, in which he mentioned that they performed oxygen consumption tests on their animals. Now the thing that occurred to me about this, is there any difference in these respiratory function tests, these oxygen consumption tests, as affected by exposure to toxic agents, and the fact that the laboratory animals that we use don't sweat? They lose a lot of heat through their lungs, and they use their lungs in a considerably different way than human beings do; and does this, in any way, impose a different sort of result when you do respiratory function tests on animals in comparison with the results you get by doing the same type of study on men?

DR. DU BOIS: The tests which can be used in men can also be used in animals, with the exception that the animals do not voluntarily go through a forced effort. But when objective tests are used that don't depend upon forced effort, the results on animals come out about the same as in man, results as to the mechanics of breathing, diffusion, gas exchange and ventilation; but these have to be scaled down, of course, to the body size. The lungs are proportionately smaller in the animals because the animal is smaller; that is, the lung weight is a certain per cent of body weight in animals and men. But as far as the metabolic functions of diffusion or oxygen consumption, these go more according to metabolic rate. But the responses in the lungs of animals, the physiological and pharmacological responses, are similar to those in men. I think the concept that animals might have different responses grew out of the fact that most of the original studies were done on anesthetized animals and it turns out that an anesthetized man has equally bad function of the lung. Anesthesia results in impairment of function of the lung. On the other hand, a conscious animal has lungs that function much like those of a conscious man. There are some minor differences in mice and rats, because the alveoli are smaller and, therefore, if the lungs are wet they have greater surface tension forces and collapse more easily, and trap air a little more easily. Some of the larger animals, particularly those with a high metabolism per gram of tissue, need small alveoli and a high diffusion surface per cc of lungs, and these are the ones that have easy obstruction and collapse of the alveoli because they are so small. Some of the bigger animals have a lower metabolism per gram of tissue and they have larger alveoli. But in general, the size of the alveoli is almost the same in small and large animals if they both have fairly high metabolism. So, in conclusion, animals and men have lungs with just about the same function, and you can use rather similar tests to measure the function of the lungs in animals and in men.

DR. STOKINGER: Would you be in favor of including this as one of the minimal criteria in these studies of space toxicology?

DR. DU BOIS: You'd have to be more specific for me. I'm not sure. Is this Conference limited to the study of animals, or does it include man? Secondly, I don't know what the words "minimal criteria" mean. Some people have been using them to mean LD-50 as the survival of an animal. When we get into the human situation, of course, we have to use physiological tests. We don't have the choice of using survival as the criterion.

DR. STOKINGER: Dr. Thomas, do you want to define for the people here what you mean by "minimal criteria"?

DR. THOMAS: We use this word here today only for the purpose of comparability of data from one laboratory to the other. Minimal criteria for biological work, so that they become comparable from one investigator to another investigator -- this is the intent.

DR. DU BOIS: I see, then I can clear this up: If one laboratory is using animals as their model for toxicological studies and they wish to extrapolate this information to man, you could do so by measuring the function of the lung in the animal and then use this as the basis for comparison with man, because we know we can't autopsy half the people or anything like that, so you have to use functional tests as your end point in man. It would be a good point of comparison or a framework in which to compare the effects of two agents. If you have an exposure to sulphur dioxide, a given level of this gas would produce a given physiological change which is measurable, numerically, in the lungs. This can be measured in an animal. It can be measured in man, and these two can be compared. So, I would say yes, in the last analysis you want to use these data in man -- this is perhaps the only basis of comparison that we have as a functional change.

DR. STOKINGER: Operationally now, which animals would you feel can be used in this respect, say to get oxygen consumption measurements, diffusion tests on them -- could you describe that briefly?

DR. DU BOIS: Well, I say there is slight difference in geometry of the alveoli, other than that the cells presumably have a similar reaction, except for some of the detailed biochemical pathways which may be slightly greater in one than the other; but, functionally, in physiology, we use all the animals that have been discussed and their lungs all react in somewhat similar ways, including man. Now criticism has been leveled at certain animals in that they have more morbidity in relation to pulmonary diseases; therefore, use germ-free animals. In man, the question is, is his morbidity more like that of a germ-free animal colony, or is it more like that of the monkeys and the rabbits at which everybody has been throwing bricks? Public Health surveys show that he is more like the monkeys and rabbits in that pulmonary diseases are endemic in man, too. That doesn't necessarily mean that the animals that are studied have to be those with pulmonary diseases just to resemble the average human. You could examine the pure

situation in which you were studying only the toxic effect on lungs without pulmonary disease or without infection. But if you want to extrapolate this to men in spacecraft, perhaps it would be more realistic to throw in a few animals that do have pulmonary disease because the frequency of respiratory infections in the United States is between 6 and 10 upper respiratory infections per year per person, so that some of the planning for space flights should be on the assumption that people will have respiratory infections. This seems inevitable.

DR. STOKINGER: Do I infer correctly that you find no difficulty from one species to another in having them adapt to these masks that they have to put on to perform the tests, even in monkeys as well as rats -- is this a problem?

DR. DU BOIS: There are various ways of doing this: You can use neckseals. You can have them in a box with the head sticking out and a collar around the neck, or you can use helmets that fit around the whole head. You can use intubation, intra-tracheal tubes, tracheotomy -- there are a number of ways of mechanically fitting the collection devices of gases onto the animal.

DR. STOKINGER: That presents no difficulty in terms of rate of respiration?

You see that in some of our animals that we have been working with, rabbits and rats and guinea pigs, with respect to getting oxygen consumption, some of the animals perform better than others of different species. I'm just wondering if that is not a problem.

DR. DU BOIS: The classical instrument for measuring oxygen consumption in animals is the respirometer which is a closed box; the animal sits in the box and you monitor the air going in and out in terms of volume rate of airflow and the gas concentration of the oxygen -- from this, you calculate the metabolic rate. This certainly doesn't require any fitting. Collection of gases from the lung may require fitting of some sort and this can be done with most animals. Veterinarians, of course, have to anesthetize animals and they use all kinds of masks.

DR. STOKINGER: Very good. I think the time is running out. I would suggest that perhaps now we would like to have Dr. Hodge and V. K. Rowe sort of summarize a little bit for us what they feel is the result of all this conversation of the "minimal criteria".

My concept of minimal criteria involves economics. Isn't that also what you mean in your minimal criteria?

DR. THOMAS: That is correct.

DR. STOKINGER: That is what I assumed you meant by minimal criteria. Could we hear from the "great accumulator"?

DR. HODGE: V.K. and I, instead of going out to a super deluxe place where they served you know what last night, sat up in the room and talked about possibilities, trying to see if we could imagine what minimal criteria might be. We will give you the benefit of this cogitation -- certainly not in the spirit of promulgating a package, but of putting up a target that the rest of the panel and the audience can shoot at. Would you agree with that, V.K.?

MR. ROWE: Absolutely.

DR. HODGE: V.K. was writing things down last night and I suggest that he read off what he has written and I will put them on the blackboard so that you will have a real target in front of you. Any objection, Dr. Stokinger?

DR. STOKINGER: No, that sounds good.

MR. ROWE: In general, it seems to me that there ought to be in this type of work, and in other types for that matter, some basic core or thread that leads through the whole pattern. This, by necessity, has to be minimal. In other words, you simply cannot do everything on everything. The numbers of animals must necessarily be limited, and all the rest of the parameters must also be limited; but there should be certain common measurements made wherever this type of study is done. I would like to start off first by asking a question that I don't know the answer to, and that is: What data are available to indicate the value or the necessity of a 24-hour day exposure versus one of 23 or 23 and 1/2? Perhaps Dr. Thomas can supply the answer. Again, I'm thinking of economics. If one is going to operate at 5 PSI, I don't think there is likely to be any practical monetary savings, because this is, in itself, a very expensive operation. However, if we are talking about ambient conditions, I think there might be a tremendous amount of savings if we could operate on a 23 or 23 and 1/2 hour day basis. Now we also recognize that there may be certain materials wherein such deviation would be very significant, and others wherein it may be completely insignificant. I would say that if there are no data to indicate the necessity of 24-hour day exposures then perhaps a worthwhile research project would be to find out. I don't know that anybody is in a position to do such a study now, but certainly side by side studies involving not only reduced pressure, but also contaminants such as have already been run, perhaps carbon tetrachloride, as a systemic toxin, and NO₂, a respiratory irritant, would be appropriate materials for such a study. There is another matter in regard to chamber operation that must not be overlooked, and that is the analysis of the contaminants. I may have missed it yesterday, but it sounded to me as though most all of the analyses were on a spot sample basis other than perhaps the monitoring of the oxygen and carbon dioxide content of the chambers. When one is studying contaminants, I believe it to be highly desirable, if not essential, to institute continuous analysis. This is so

simple in most instances today, that I see no reason why this shouldn't be done. The tonometer method for sampling on a spot basis is superb, but it does not substitute for continuous analysis. We've done quite a lot of this sort of work. By employing the techniques presently available, small samples, just a few milliliters of gas are quite adequate for almost any analysis, with an accuracy down to fractions of a part per million. Dr. Hodge, what do you think, shall we stop at various items, or should we run right on through the group?

DR. STOKINGER: I think we might take up some of the items, otherwise, they get lost in the shuffle. I was wondering as you were talking, whether the audience is clear about the advantages of the 23 versus the 24-hour exposure. Would you talk about this, Dr. Thomas?

DR. THOMAS: You ask me about available data for comparison. The only thing that I can think of are the studies on ozone which were done by Capt. Siegel and the ones which were done by us, and there is nothing else so far as I know in the literature. You made a very valid point there. If we do exposure work at 5 PSI, we have to have an airlock. You must operate uninterruptedly, otherwise you will be pumping your animals up and down, and that would really be a physiological stress. Therefore, in the case of space cabin work, unless space cabins go to 15 PSI normal air composition, right now we have no choice. But it is very true that a classical experiment should be set up. I personally think that, knowing how toxicology works, sometimes you lose your concentration inadvertently, and whether you open up that exposure chamber for ten minutes or fifteen minutes and do a quick cleaning job, I don't think it would make a lot of difference. I think that the way that we use "continuous exposure" in our terminology means that your organism is continuously being taxed with a toxicant. You get a summation of interest type of damage, and you do have a short recuperative period, but I don't believe a ten or fifteen minute interruption in gassing will make much difference.

DR. STOKINGER: I might speak about that a little bit. The only time that any interruption would make a difference would be in the beginning of an experiment in which the toxic agent was a respiratory irritant. Then, of course, other factors take over, as I'll illustrate a little later in another connection, such as the development of tolerance. But after the exposure has been in progress for a day or two days, a little interruption is of no consequence. This is certainly true of materials that accumulate -- or have effects that accumulate -- in contradistinction to the materials, so I think there would be a very small chance of effects from an interrupted exposure after the first day or so. Is there any other comment?

CAPT. SIEGEL: May I make one little comment? All of the Navy work, at ambient now, has not been continuous in a true sense. We have opened the chambers every day. We clean out the chambers once a week and

our "down" time has been 2 per cent. So it is not continuous, if you want to take a strict interpretation of continuous exposure. But again, if we go to pressure, we would not be able to run it except continuously, because it might take a long time to bring the animals back to ambient pressure.

DR. THOMAS: Also, Jac, you pointed out yesterday that we have some discrepancy in results here, and I wonder what the reason is. Would it perhaps be that ours were all truly uninterrupted, whereas yours were, as you say, with a 2 per cent down time? In ozone and NO₂ we approach the same comparative levels, but you feel that there are some differences there.

CAPT. SIEGEL: We are beginning to talk on a different subject, but I think we are talking about the enzyme systems and the changes that you find -- some marked changes -- in some of the enzyme systems measured in animals exposed to extremely high concentration and which one would expect. But when you get down to trace contaminants, you won't see these changes.

DR. THOMAS: I was thinking of the death rates primarily. You run ten parts per million?

CAPT. SIEGEL: No, five -- less than five ppm for NO₂ and less than 1 ppm for ozone.

DR. STOKINGER: V.K., did I not hear -- what was your statement about the sampling methods? I thought you were going to say it was necessary to have continuous monitoring. Did you say this or did you not?

MR. ROWE: I did.

DR. STOKINGER: I guess I missed it.

MR. ROWE: No, I meant to say that I thought in present day technology there was no problem of running continuous sampling and recording of concentrations. This is very enlightening if you want to (and we do commonly) use other methods for spot sampling just to check your monitoring equipment and to see that everything is operating all right. But the instant something breaks or goes down you have an immediate, or within seconds, alarm system or some such arrangement that will immediately alert you. Otherwise, if you are relying on spot sampling, you never know when things go down.

CAPT. SIEGEL: May I make a comment on sampling? We have for years now sampled both by continuous monitoring, automatic injection of samples to a chromatograph or infrared instrument or similar constant monitoring system. Simultaneously we have run bubbler samplers or some other spot method. We have had terrific fluctuations in the spot hand samplings, variations from day to day and from hour to hour. Yet, when you measure concentrations by instrumental continuous recording techniques, you get a beautiful straight line.

DR. STOKINGER: There is a very good reason for using continuous monitoring devices, and that is that the response from many of the things that you are testing, particularly pulmonary irritants like NO₂ and ozone, and other things that are coming in for testing, the response is an acute one, a direct one and an immediate one, and is dependent upon peaks, and if you have to take a spot sample, you may miss these peaks; and that's just the thing you want to observe, because it may just turn out to be that this may constitute the difference between results that seem to be discrepant in your work, Dr. Siegel, and yours, Dr. Thomas. There may be some incidental peaks along the line that the animal has responded to and didn't have the tolerance built up to handle it.

DR. THOMAS: May I make a comment? There are some other ways around this continuous monitoring also. If you have your contaminant feed monitored and your airflow rate monitored and alarmed, then you will avoid these peaks also.

CAPT. SIEGEL: I don't agree with that at all. I think you can have what appears to be a perfect input -- (we do this all the time) -- we monitor the input and the airflow, and the chambers are alarmed. Yet, if you have a leak in the system or some other discrepancy, you can have the same input, the same airflow and you are not going to get the same concentration in the chamber. I think you've got to stick to measuring the concentrations in the chamber.

DR. STOKINGER: The middle of the road answer to that, of course, is that I think both of you are right. If Dr. Thomas runs his toxic agents in at a high speed, then fluctuations, of course, don't occur so readily as when there is a sluggish atmosphere in the chamber for the absorption to take place. To give you some examples of this: In a small chamber, at one time, we were working with hydrogen peroxide. This is one agent that absorbs on the hair and body of the animal. We were running this material through at a fairly slow rate in a little 20-liter jar, and when an animal would die, the concentration would go up because the animal was not now absorbing it in the way he did before, when he was alive. These effects, of course, are real. One way to get around it is to maneuver your rate of flow through the animal chamber at such a rate that the small absorptions do not occur. Shall we go on to the next point?

MR. ROWE: Only one thing perhaps on sampling: In continuous analysis generally you may have sampling leads that go for some distances. You've got to be very careful about the composition of those leads and the continuous flow of the material through them. In other words, if you are going to use a lead for spot sampling or checking a monitor, it should be set up so that you maintain a flow continuously through long leads and then divert through short leads to your spot sampling device. If you don't, then you may have absorption in the leads and erratic results.

DR. THOMAS: In defense of my own position, don't you think the size of the chamber has a lot to do with it? Our domes have a tremendous buffering capacity. So we don't have to worry about that, but most people will be working with small chambers and there it is very critical I think.

MR. ROWE: The next item I have is this matter of "standardization" and I heard Capt. Siegel say this morning that he would like to know what species and how many to use and that is a big order. I'm not going to suggest what species a person uses. I would like to see the number of different rodents minimized, and at least three species used. I think the scientist ought to be capable of making his selection of the species depending upon the type of action the material has. There may be times when he wants to concentrate on the cat, the dog, the rabbit or perhaps some other species. Certainly there ought to be some, maybe one or two species, that are common to all studies involving multiple species. I would like to see the dog and the rat included in all basic studies, simply because there is so much background information on them. Perhaps the monkey would be the next choice of animal. I would open this up for discussion; the same applies to the numbers of animals. I think it depends upon what you are going to do, what you want to accomplish in the end, how many animals are necessary. For example, if serial sacrifices are part of the program, then you certainly have to start with larger numbers. The main thing is that you have groups of animals sufficiently large so that statistical analyses will mean something. If one out of two animals dies, I don't think the statistician can do well.

CAPT. SIEGEL: We reviewed all of our data just before coming here and we find that the following order of sensitivity in our animals exists in the five species: guinea pigs -- most sensitive -- then rats, rabbits, monkeys and dogs. Then in the order of reliability, meaning that the controls are good and they are reproducible, were: dogs, guinea pigs, rats, monkeys and rabbits. I just throw this out.

DR. MAC EWEN: I was just wondering, are you inviting comments from the floor?

DR. STOKINGER: I think if it is pertinent, surely. We want to settle these questions as well as we can, however we can, whenever we can.

DR. MAC EWEN: There are probably a number of people who would like to make comments from the floor as we go along here and I didn't hear any mention made that they would be invited. First, I would like to make a comment on this continuous versus intermittent batch sampling technique. There are many excellent pieces of instrumentation for continuous monitoring. The difficulty is for the experiments which we described yesterday, continuous exposure 5 PSIA oxygen, in that we were exceeding the sensitivity of most of these instruments and once you start getting into sample splitters, you are also increasing your error problems. We were sometimes ten or a hundredfold

higher than the maximum sensitivity of these available continuous monitoring instruments, and this is one of the tradeoffs. When you are working with trace level contaminants, there are some excellent continuous monitoring devices which we have available in our laboratory.

DR. STOKINGER: It depends upon the concentration and the sensitivity of the available instruments.

MR. ROWE: On the selection of animal species, I have one note that I failed to mention; I think one of them ought to be a species that has a metabolic pathway as close to man as we can find. This may mean some work on man very early in the game, but we should find and use the animal that has a metabolic pathway similar to man. I think we will be one step in the right direction.

DR. THOMAS: I would hesitate to generalize quite as much as Jac did in the order of sensitivity, because I think it would depend upon the agents. I think that one of the things which came out of yesterday's results in the 5 PSI oxygen plus contaminants exposures was that we found a diversity in sensitivity between the monkey and the dog from NO₂ and ozone. Did you ever observe this -- reversal of sensitivity between dogs and monkeys from NO₂ to ozone?

DR. STOKINGER: I think I can offer an explanation of this: One of the reasons that I had in mind when this information first came to me was that the monkey is capable of developing a tolerance at a much faster rate than the dog, and why this doesn't occur in the NO₂ experiment is this -- that NO₂ does not produce a tolerance as readily in animals as the ozone does. Now if that's the basis for it, the enigma would be explained.

MR. ROWE: The next item that I have down was this clinical chemical work and here is a place, I think, where some standardization could well be brought about. If people are going to compare results of various tests, one laboratory to another, I believe it is highly desirable that they have some sort of a communication between them. For instance, referee samples should be sent from one laboratory to another to be sure that even if each laboratory is using the same method, they are getting the same results. Now if this is not done, I don't know how you can possibly correlate your figures when you get through. Now, what tests should be run? I'm not going to say. Here again, there is a certain core that I think would be highly desirable to include in any study; but, again, what additional work is done will depend upon the particular experiment and what the objectives are, in the opinion of the investigator. But, certainly, again, we should have a core, a few tests, and well standardized.

DR. STOKINGER: Could we possibly settle it -- this sort of basis of standardization, that certain minimum routine procedures should certainly be

standardized; like protein and urea nitrogen, blood counts, and these things that are commonplace, and then this leaves the way open for individual initiative. In this way we don't intend to standardize and stigmatize initiative and development of new methods.

DR. HARRIS: I would like to make a comment. A standard procedure among hospital clinical laboratories is the submission of standard knowns. Each hospital is requested, and is expected, to return its analyses of these particular mixtures. The standard may be a combined serum sample or synthetic mixture. The methodology of various laboratories may not differ, but the results from many laboratories have been found to differ considerably depending upon the adequacy of the people who are running them. It is not merely a question of selecting our methods, because in many instances they bring similar results, but the performance of each laboratory should be considered individually. I would suggest that in considering which tests are to be run, a procedure similar to that being used among the hospital clinical laboratories should be considered. Do you agree?

DR. THOMAS: I certainly do, and I think I would like to see some quality control procedures introduced in every laboratory that gets involved in this work. I understand that the clinical chemists' association may make standard samples available through one of the drug houses for this purpose in the near future. Mrs. Pinkerton, do you know whether this will be available?

MRS. PINKERTON: Yes, but I do not know which company will supply it.

This happens to be a project of the Ohio Valley Section of the American Association of Clinical Chemists, but I am sure that other groups could also do this sort of thing.

DR. HARRIS: Several years ago, a number of samples were sent to various laboratories and analysis requested. It was amazing how much variation was reported for components like calcium and glucose, although similar procedures were used by the different laboratories. One thing that we have in our favor is automation. The more determinations that can be automated the better off we are for comparison of results. Automation, of course, requires large numbers of samples, or it becomes economically unfeasible. A commercially prepared freeze-dried serum could be standardized and submitted to different laboratories. The analytical laboratories do not have to know the source of the preparation. The preparations can be made deliberately abnormal. This should be one area of standardization that I think we should agree upon. It is not the methodology as much as it is the results that come out that is important.

DR. STOKINGER: Dr. Harris, what sort of a schedule would you suggest for checking this out? Would it be on a yearly basis, or every two years?

DR. HARRIS: I think a yearly basis would be all right. More often, it would be pushing it.

DR. STOKINGER: Does that sound agreeable?

DR. CARSON: (from the floor - Carson, Food & Drug Research Laboratories, New York) At the recent toxicology meeting that took place at Williamsburg, our laboratory presented a paper on the technique for developing internal quality control standards for the laboratory. Before I got here, Dr. Thomas and Dr. Back asked me to comment on it, so, for what it is worth, I would like to point out that there are a number of errors that can creep into a clinical laboratory, some of which have been alluded to in the discussion that just took place. But one of the major problems that must be worked out is the human error that takes place in the laboratory. Among the techniques that we have used, and it bears out, because the Hyland serum was just mentioned and this is lyophilized, is to bleed out relatively large quantities of blood for rats and dogs and we then split these samples so that we can run a complete series of determinations on these split samples. These samples go into the freezer and we take just enough material so that we can run out this series. We do this in every day's run so that we have established for the laboratory quality control range, and we can pick up in our system human error as opposed to changes that have occurred in the instrumentation. Reference has been made to the auto-analyzer, which is a unique and excellent instrument, but it has some internal problems of its own. The laboratory that elects to use an outside standard system, such as Hyland or Versatol, as a single expedient may run into certain problems which we have seen, because you have the problem of not having a protein base. We have elected to use both standards. Any time when we are using monkeys or dogs, miniature pigs, rats, rabbits, we continue to throw in standards each day so that we have a Versatol or a Hyland, plus the internal standard for the laboratory, and all of this is done on a blind basis. Our technicians report data which goes into an IBM system and they are reporting values that are read off the colorimeter, or read off the auto-technicon. They are reporting raw data. They have no idea what this will convert to mathematically when the IBM makes the necessary conversions for absolute values. We have found this to be particularly valuable in the handling of the problems that come from concurrent controls, each animal serving as its own control, and it has given us an excellent basis for comparison of data on a day-to-day basis, or a period-to-period basis, in following chronic toxicological environmental exposures or feeding studies.

DR. HARRIS: I agree with you whole-heartedly. In other words, every laboratory should have its own internal control setup. Blind sample run-throughs, standards of pooled serum, pooled urine samples, frozen and

maintained for just such purposes. I think the point of discussion here was a coordination between laboratories so that we could know that what one laboratory is reporting is similar to that which another laboratory is reporting. You may work out very well on your internal standards, but your results may not agree with somebody else's laboratory.

MR. ROWE: Thank you. Let me go on to the next item.

DR. BACK: (from the floor) One question to the panel on the lab methods. This is one of sampling. We were talking about how many times to sample and so forth. When you have a long-term experiment going, 90 days, what about these samplings of rodents? We like to do blood work on rodents, and what does the panel think about pooled samples? This is a big problem. Everyone realizes this. What would you, any of you, pool? -- Five rats and call it a group and use pooled blood? What's your feeling on this?

DR. STOKINGER: Who wants to answer that? Do you, V.K.?

MR. ROWE: Sometimes this is your only alternative, if you need to run certain analyses which require more blood than you can get from one rat or one mouse. We frequently do this, but we don't like to pool blood unless we absolutely have to. We think we can get valid data from this procedure, but one has to obtain a set of control samples in the same way.

DR. BACK: I understand that we need controls. What I am getting at is, if you were to sit on a panel on a drug board, would you accept the data?

DR. STOKINGER: No, I wouldn't. (Laughter) If I could just answer that question --. We have been considering this, of course, for many years and we have set upon the following procedures where rodents are concerned. We use no fewer than ten animals and the reason for this is that the rat varies so much from animal to animal that you cannot make a decision from the control values which, in turn, vary, particularly when you are dealing with the minimum responses. If you are dealing with a life or death phenomena, there is no problem in using pooled samples. But the things that we intend to look for are small differences and where you have to review it statistically, numbers no less than ten are required. We have found that you cannot make a decision in most cases unless you do.

DR. TOKITO: (from the floor) I'd like to back up Dr. Harris' remarks. I am Tokito from NASA Ames, Moffit Field, California. We recently contracted out some hematology work, and with a simple thing like a micro-hematocrit, the clinical lab versus our results were different by three or four units. So we did a quality control and found out that our instruments were not giving the right hematocrit, so these are methodologies that are worked out. The centrifuge wasn't rotating at the same speed, say from one lab to the other, so the results were different. So this, I think, is very important.

DR. STOKINGER: Thank you. I think we will have to move on. We are way behind the schedule. Could we go on to another topic? Or have you completed the first one? I guess we have some more comments here on species selection.

MAJ. REYNOLDS: I don't want to retard the progress of the meeting, and I apologize for regressing here, but I didn't know we were going to move away from species selection so quickly. I think in the choice of an analog for men that this deserves a great deal of consideration on the part of this group. Certainly for various types of screening activities I think lower animals can be used very successfully. But the ultimate purpose is not to study the animal itself, I do not believe. Certainly for various types of extermination processes, in the interest of our economy and so forth, and to prevent various types of destruction on the part of lower animals, maybe we want to study the animal itself. But as to the nature of the work that this group does, I think that there is an attempt to extrapolate to man, or at least we lead people up to a precipice and let them extrapolate. But I feel that we must consider that man usually is not inert, he does not sit still. He is acting upon his environment continually, and I think that when we do not seek animals and select animals that are capable of emitting certain types of responses that approximate those of man, then ultimately we will not be able to draw the best inferences that we are capable of drawing. So I think that in any process where we are studying something that might pose a high risk to man, we are compelled to go to animals with higher cortical development. There are many differences of a physiological nature that I could explore, but probably I am not competent to do so. Yet, I certainly know from a behavioral point of view that there has been a degree of cortical development in the primate -- subhuman primate -- (although this may not be the preferred terminology, we nevertheless use it) monkeys, chimpanzees, baboons, orangutans and other primates, which enables them to do things that are similar to what man can do; again, as I said yesterday, particularly in terms of manipulatory behavior. So I would like to see some questions or some thoughts on this, and be challenged if necessary, because I think that these are important questions. Certainly it may be more convenient, it may be more economical, it may even be more statistically sound to use lower animals and achieve a larger number, but whether or not, in the final analysis, we are asking those questions of lower animals that must be ultimately asked of man is an important issue.

DR. SCHWARTZ: (from the floor - Grumman Aircraft) I'm a physiologist. I believe it is important in studying agents which affect the central nervous system, naturally, to have animals with a cerebral cortex and chord similar to man. However, since many of these agents act on various other systems, I think it is less important, other than the central nervous system, to use these higher primates. I would like to point out a very interesting species which has been largely overlooked and can give some very valuable data -- the nine-banded armadillo. This is a useful animal where genetic effects or genetic variations can be very carefully controlled. As some of us know, the nine-banded

armadillo exhibits a specific polyembryonae. It always has absolutely identical quadruplets. So when you work with this animal, you actually have one original and three carbon copies; so that if you do genetic control and the host factor needs to be controlled, you can take two of these from one litter and experiment, and take the other two and use them as controls, and you've eliminated the variable host factor. These are not rodents. They are mammals and, in spite of the fact that they are mammals, I'll tell you when I applied for my license to use them I was informed by the State Commission that I didn't need a license to work with rodents; but they are mammals and a very useful species. I think you should tailor your species or split your species, according to the type work that you are trying to do.

DR. STOKINGER: I think we will have to continue with the other points we have addressed ourselves to because the time is short.

MR. ROWE: The next item I had down is "hematology".

DR. HODGE: Before we leave this subject of standardization, let us put one more word down that we talked a lot about last night -- "vocabulary".

MR. ROWE: What needs to be done in hematological examinations? We are doing about everything in the book. However, I have heard the hematologist say we shouldn't do anything more than a microhematocrit and a white cell count. If we see something abnormal in these, then more extensive work is indicated. Generally about 99 per cent of the rest of the work we do routinely is not revealing. So, as a minimum, these two parameters are what we would suggest. Dr. Hodge and I talked this over last night and we realize that there are lots of other things that can be done. How far do you go? I'm certainly open on the subject, maybe other people have comments. Is there any conversation to this point, Dr. Stokinger, from the audience or even the panel members? How do you feel about it, Dr. Thomas?

DR. THOMAS: I think that the only time when I would prefer to have more done is on establishing baselines on the larger animals. I want to make sure that they are healthy before we put them into the chambers.

MR. ROWE: I didn't hear anything yesterday in regard to analyses, chemical analyses, of concentrations of material in the blood, or in expired air. It would seem to me that this would be extremely valuable data to get, at least on the primates, and perhaps on the dogs that are used in any of this long-term work. Blood concentrations, I think, are exceedingly important, and they can be correlated very nicely with the expired air concentrations.

This is perhaps a little out of the line of the regular pattern, but in regard to this tradeoff concept, it would seem to me highly desirable to take a few animals in each experiment and study them very, very intensely and then use larger numbers of animals to build up your statistics and so on.

There are many, many things that can be done on a few animals with a minimum amount of time expended, and cost for that matter; and a thorough work-up might very well give you a lead that is significant. It would be prohibitive generally to do this on the bulk of the animals.

DR. STOKINGER: I might just comment on the blood analyses. These compounds that will be mostly under study -- many of them will react with protein components and then no longer exist. For example, ozone and NO₂ cannot be detected in the blood, and many of the other respiratory irritants are so reactive that they no longer exist as such. Of course, another factor is that the levels of concern, practical concern -- they may be in very small concentration in the blood -- even those substances that we may be studying that do not react and destroy themselves through this reaction.

MR. ROWE: How about the contaminants that may not appear in space capsules, but which may appear in other confines, such as the solvent vapors that Capt. Siegel was talking about yesterday? It would be extremely valuable to know how much was present in the subjects as well as in the atmosphere.

DR. HARRIS: I think it is an excellent idea and I would like to have one other fluid, and that is urine -- to see how much is excreted, either as a metabolite or in a free form, so that a complete picture of intake, blood levels and, finally, output is obtained.

LT. COL. REEVES: You might as well add feces, saliva and tears to this. I think it is too much.

DR. STOKINGER: We are talking about minimal criteria here. We don't want to include an entire treatise on the subject.

DR. BACK: (from the floor) We did do this in our early studies with UDMH and with decaborane, and we got good blood tests; unfortunately though, we couldn't pick up anything even after the 90 days. These animals didn't have any that we could measure. Many times I think it is a minimum criterion. I think you are absolutely right that it is always difficult to do.

MR. ROWE: I think you may find that you have appreciable levels early in your experiment, but as your animal adapts its metabolic capacity to handle these things, you will find this goes down. This is a real important point when you are thinking about chronic or long-term exposures. Does the animal have the capacity to change or adapt metabolic pathways?

DR. BACK: We would like to tag some of these materials and serially follow them, and certainly urine is a good way to do it, but then you've got to add another parameter to your chamber. You've got to have good metabolism cages, and this is a rough row to hoe too.

MR. ROWE: That's why blood sampling would be desirable, it is so easy to do.

DR. GETZKIN: (from the floor - North American) I question the tagging or the following of these compounds in the circulating fluids, and I agree with Dr. Back that it is more important to determine the locus of action rather than to determine its presence in the circulating field.

DR. STOKINGER: Thank you. May we continue? Another comment?

DR. CARSON: I would like to add something. We have really hit on the crux of it, I think, right at this point. I would like to say that in listening to many of the talks yesterday I had the feeling that the engineers had run ahead of us, and that, as toxicologists, we were still in the business of bottling animals so that the pathologist had something to look at. I would like to go back to the point that we had here, a proposal by V.K. to look at several animals intensively. This is a program that we have tried to do in our own laboratory, and we've got some support from the AMA in this in tobacco work. We are attempting to assess a total cardio-pulmonary picture in small animals. Dr. DuBois is perhaps the man who can discuss a lot of these things much better than I can, but I would like to make a plea for an intensive evaluation of a number of animals to gain the maximum physiologic parameters and to determine the changes as they occur during the environmental exposure. It seems to me that we keep talking about tradeoffs and we keep talking 90-day, and, perhaps like all other areas in toxicology, we may end up with the one-year and two-year studies. But an intensive evaluation of the responses of the animals, as they occur, will give us some indication of tolerance development. It may give us some indication of the metabolic pathways and may, in fact, enable us to cut some of these exposures short, because we have some index of where we are going, how the animal is handling the product, and where the differences between species lie. Further, some indication of the mechanism of action whereby the effects actually are manifested. We continue to look at tissues, and this is our ultimate, this is the only thing we have; but I would like to make a plea for intensifying the physiological evaluation during exposures, not before and after.

CAPT. SIEGEL: If you are going to run a continuous exposure, all of these physiological measurements are going to take time; and you are not going to be able to continually expose these animals if you are going to do a daily or weekly examination, unless you have your personnel enter and do their work in the chamber.

DR. THOMAS: May I have one quick word here? One of our biggest problems at altitude (100 per cent oxygen 5 PSI) is that we cannot find any data in the literature on how the pulmonary function tests change while at altitude. We have data on humans before and after exposure, even as long as 30 days stay at altitude, but nothing while they are at altitude. The other thing which bothers me about this is that I don't know what the rate of absorption is of various toxic agents in the lungs at altitude. Some of these measurements we've got to do, and I'm sure we'll never find our own answers unless

we do it. And you just have to do it while the animals are at altitude, so it's got to be done during the continuous exposure.

DR. CARSON: Jac, I'd like to make some response to what you have pointed out. I agree that it is very difficult, but I don't think this is an insurmountable problem. Certainly veterinarian surgeons at the Wright-Patterson installation have learned how to put catheters in just about every orifice and tube available in the animal, and the techniques of modern telemetry have not really been used as they should have been. If the engineers can produce the kind of dome situation that they have at Wright-Patterson, I'm sure that the electronics men can miniaturize many of the things that we need to carry these out. I think that it is not an easy problem, but, on the other hand, I don't think it is an insurmountable one.

CAPT. SIEGEL: I agree. I was talking about the classical physiological work that we know of today. But we are in the early stages of telemetering physiological data, and I think we've got to extend this area to where we don't really monkey and interfere with the animal's exposure.

MR. ROWE: Two last items, and I'll handle them together, because it is a subject that Dr. Spencer brought up yesterday in the minimum pattern for pathology and organ weight studies. I'm just going to reiterate what he said -- gross autopsies should be done by a pathologist and appropriate records kept by him and that organ weight and microscopic studies should be done on the lung, liver and kidney as a core and then include other organs as indicated. Experience and data show that the core of studies will detect a very high percentage of those materials producing adverse effects. Remember, this is only as a core.

DR. STOKINGER: Any comments on this?

DR. CLAYTON: (from the floor - DuPont) V.K., I wonder what you mean by "as indicated"?

MR. ROWE: My feeling is that it should be at the discretion of the pathologist doing the work. He should be competent to know what other systems he may want to sample. In other words, if he is dealing with one kind of compound, he may want to sample one system; if he is dealing with another kind of compound, he may want to sample another system. Ordinarily, as you well know, in much of our work we are taking 25 to 30 tissues, most of it to get negative data, we admit that. But with certain types of compounds, you know very well that you want to center on a particular organ; with others, it may be another.

DR. CLAYTON: It always bothers me that a priori some decision is necessary to do this and I'm not quite sure that we are always sure that the decision is correct. That in one case the lungs, liver and kidney may be the

routine, or the standardized choice; but, nevertheless, how can a priori now before anything is known about a compound, can anyone make a choice that certain organs ought to be eliminated?

CAPT. SIEGEL: Let me illustrate with an example. When we were doing triaryl phosphate, we definitely decided from the beginning to do the sciatic nerve, to look for demyelination. This is the type of thing we have in mind.

DR. CLAYTON: By that time, the phosphate acted on the myelinated nerves. I'm wondering about those materials about which we have no knowledge.

DR. STOKINGER: I think we will have to turn down any further comments on this minimum criteria and turn our attention to the second topic which is a little belated, but it has already been touched upon in many aspects, and that is the tradeoffs. I would like to pose a question to Dr. Reynolds here on my left of a tradeoff that he talked about in his speech yesterday, and I have phrased it this way -- Does the potential of behavioral measurements on chimpanzees compare well for evaluating sensory effects in man; that is, of irritation and discomfort, that can be made up for in man's speech which the animal is deficient in? Do you think that the use of the chimpanzee is a good choice of animal in this area of toxic response?

MAJ. REYNOLDS: First of all, I would like to clarify one thing for the person who submitted the question. They say "does the potential of behavioral measurements on chimpanzees compare well for evaluating sensory effects in man, that is, irritation, discomfort". These I would not call sensory effects. I could call these affective states, because really these are not concerned with the sensory modalities as such. Directing myself now to the question as clarified, I think it would be absurd for me to say that any behavior measurement on animals makes up for the speech deficit; because this is the one thing that, I think, enables man to do the things that he does in terms of word rather than deed. Certainly the chimpanzee is highly capable, there is no question about that; but I think that no behavior measurement which we now have in any way can make up for the deficiency of speech that we find in animals. I would be very hard put to try to justify that.

DR. STOKINGER: Any other comment on this?

MAJ. REYNOLDS: I might just say this for your interest. There was a chimpanzee that some of you may know about -- Vickie -- that was raised by Keith and Cathy Hayes, which they discussed in their book, "The Ape in our House". This animal did learn to say certain words like "Mamma, Poppa, cup, table" -- things like this. But to arrange these in a sentence, in some coherent order, just doesn't come about. That's just an afterthought.

DR. STOKINGER: Do you think there is evidence of training these chimps to a high degree? Do you suppose we could substitute them for man, make the first drop on the moon, take a sample and come back with it? (Laughter)

MAJ. REYNOLDS: We've been pushing for that for a long time.

DR. STOKINGER: I would like to suggest a possible tradeoff that I am sure has been in the minds of many individuals in the audience and that I have had in my mind for a long time, and I have spoken to all of those people who would listen to me about it, and that is: What about the tradeoff of determining the toxicity of hardware and men versus these animals that we are using in synthetic atmospheres? Now there are two very basic reasons why I think that this should be given a very close look; and, if I can get to the board, I want to put on, in a graphic way, one of the most cogent reasons that I can think of for resorting to this exposure of hardware to human volunteers. One of the reasons is the nature of the exposure. If you make a simple diagrammatic representation of time along here and response over here, the present studies have somewhat of a dosage response curve like this, more or less constant. (Illustrates) On the other hand, if you take the hardware that is known now, the design criteria that is known in great detail down to the last bolt, throw these in the middle of your dome chamber, and then get ten good men to volunteer to stand around this, under the conditions of exposure, both ambient and 5 PSI, what do you find? You find that the exposure is entirely different from the present method used for animal exposure. There will be a gradual buildup, and it will be very gradual, and all the more so because I understand they are now contemplating the removal of many of these contaminants in the air by means of carbon filters. The point I want to make here is that this will lead to a different type of a response from a gradual buildup, opposed to when the man gets a slug right away as it is done necessarily in the synthetic atmosphere. In case of pulmonary irritants, another prominent factor occurs, and that is this: As soon as these pulmonary irritants get down into the respiratory tract, another phenomena sets in, known as tolerance, and this sets in as early as the exposure does; and as the exposure increases, the tolerance increases. So you have a condition here that is not seen in the animal that gets the synthetic atmosphere in maximal concentration all at once. Now the next cogent reason for doing this is the type of synthetic atmospheres that must be reproduced. These mixtures can never be approximated by synthesis, and the reason was clearly shown by the effects of the automobile exhaust that you saw yesterday. As time went on there was interaction, and this, of course, can occur here where we have all sorts of different chemicals that can interact and will be absorbed at different rates by carbon filters; and, as Jac knows, all these functions, absorption processes, go on as the absorption becomes complete for one substance, something else takes its place. That substance which was absorbed last month is now no longer in the absorbing medium; it's back in the air through replacement. So you are continually changing atmospheres. For these two reasons,

I would like to suggest this as a very good possible tradeoff for the animal work. I do not mean to replace all the animal work. Keep the animal work to work out the details of metabolism and mechanism, and possibly for screening certain things; but I surely would like to see two of the chambers that are now in use, start right in on this, where you can get your answer directly. You can terminate the exposure at any time if you have good medical surveillance.

DR. HARRIS: This is a point under discussion at this time, and our position, you might say, is that it is rather difficult for a man to get into the spacecraft for the first time and test it out. The materials have been tested in a gross way, but you have a completely different environment in a spacecraft once the materials are in place and operational. There are several possibilities of approaching it. One is to outgas the spacecraft, taking the outgassing material and subjecting animals to it. Another way, of course, is to take a complete spacecraft, run it with its systems going, and, with very careful medical monitoring, observe the men inside. In this case, whether the chimpanzee can talk or not talk becomes very important. The man inside can tell you yes, he feels nauseous. The rat can't, neither can the chimpanzee. The man can tell you he has a headache; he can tell you his vision appears blurry. At some point, man has to be introduced into this atmosphere where he can be observed before he is in orbit.

DR. THOMAS: We have been very anxious to get hold of a real space cabin, once in our lifetime. They are just too few and far between, and you can't lay your hands on them. What we are planning to do is to use a life support system, for example, and give it a clean bill of health by maintaining one of these domes for prolonged periods, and by putting in the dome, artificially, some of the known "problem" gaseous products to see how well the filter operates. We will have animals in at the same time.

DR. STOKINGER: But you do not have the human being in there.

DR. THOMAS: First I prefer to do animals -- at least until we know where we stand.

DR. STOKINGER: This doesn't take care of the man, because animals contribute different gaseous materials to the environment as I understand it.

DR. THOMAS: That depends upon the diet.

MR. WILLARD: (from the floor - Honeywell Aeronautical Division) Honeywell supplies the stabilization control system for the Apollo vehicle. I'm an engineer, a chemical engineer, and have been following this Conference with a good deal of interest so far. The suggestion made by Dr. Stokinger is something that will be implemented, as I understand the plans, for the Apollo program. Animal testing may be of considerable value in answering long-term

questions. But we, as engineers, have already decided what is going into the spacecraft. The system is locked in now, they are being built. There are no choices, except very relatively minor ones. As I understand also, one of the early Apollo systems will be a man-rated test for 30 days, for some period of time anyhow. I haven't heard yet what the plans are for examination of the occupants of that craft. I suspect that there will be some preliminary testing of the atmosphere with all systems going. But I think, in essence, what you have suggested is being planned for Apollo. What we, as engineers, would like to know is: What do you, as medical specialists, need from us to answer our questions as to what criteria we should use today for excluding materials from tomorrow's spacecraft?

DR. THOMAS: This is a subject of tomorrow's session.

DR. HARRIS: I would like to hold off on that until tomorrow.

DR. DU BOIS: A proposal has been made at Wright Field to get a spacecraft and put some animals in it and see if any of the gases coming off are toxic. Now I believe that this might be the best way to do it, because I'm not sure that you can analyze all the gases that come off a ship with sufficient accuracy to tell whether there are certain toxic contaminants -- traces would be hard to detect. It seems to me perhaps this would be one way to answer many of your questions very fast, just to get one of these crafts and do just that. What's wrong with that as an experiment?

DR. THOMAS: Nothing. We would love to do it, but you can't get one.

DR. HARRIS: They are horribly expensive. However, during the course of the checkouts, outgassing studies are made to determine what can be found and, also, what cannot be found. It would be very nice perhaps if we could go that route. The spacecraft will be subject to decompression and there will be attempts made to identify the off-gassing substances. The matter of putting animals into it is another question entirely. There just are not any spares.

DR. THOMAS: That's right, but the problem still remains. If we are very lucky in our analytical efforts and come up with 150 compounds, and nothing is known about toxicity of 70 per cent of the gas-out products, what then?

DR. HARRIS: That's a good question.

DR. DU BOIS: I thought they were making ten of these Gemini crafts, aren't they? -- What do you mean they can't be had, then?

DR. HARRIS: Each of these craft is undergoing testing for systems testing purposes, and each of these craft during systems testing is being

outgassed and the outgassing components determined. Here are some of the materials which were desorbed from charcoal in a Gemini simulated cabin run. We had totals as follows: water, 46 per cent; CO₂, 1.36 per cent; (this is a run of roughly about four hours) we had some CO which was high, 5.96; oxygen, 4; argon, 3.3; chloroform, 1 per cent; methylene chloride, alcohol, .16; nitric oxide, .015; Freon 11 gives us .23.

DR. STOKINGER: We will have time for just one more question before we break.

MR. PEARSON: (from the floor - Lockheed) I'm not an engineer. I have just one question. Perhaps it could be answered after the break. What are the minimum criteria for doing toxicological studies on man. If necessary, we could interpret and extrapolate this then to animals!

DR. STOKINGER: I'm afraid the answer to that is a subject for an hour's discussion at minimum. I'm afraid we will have to hold that until after the coffee break.

DR. CULVER: (from the floor - Aerojet-General Corporation) What is the purpose for doing toxicology? I assume it is in order that certain kinds of decisions can be made. Therefore, you need timely information. If you are going to pick up a spacecraft and expose people or animals, or analyze the atmosphere in the spacecraft, as our engineering colleague already told us, the spacecraft has been built. It's all locked in and, therefore, the toxicologists are not providing timely information. We have to devise some system that will allow us to help the engineers make selection of materials or systems that are going to be used. I think that some of the things that you are getting to, Dr. Stokinger, on board, of trying to represent what we can anticipate in the future and do our toxicological studies based on that, is a more meaningful way to go than to fool around with spacecraft cabins that are already in existence.

DR. HARRIS: I would like to hold the answer to that again until tomorrow, and I am going to discuss that aspect of it. We are locked in, there is no question about it.

DR. STOKINGER: Do you have a quick answer?

COL. WHITE: (from the floor - Air Force) The engineer is right. We must try to give them criteria. This is a classical toxicological position; but I think we also must face the fact that toxicology must take on a new regime and it follows the thing you suggested here. The man who is going to have to make decisions during flight (which admits failure in having given the right criteria) where problems are evolving, must have the criteria during flight, so that man and the crew can do something about the situation before he has to abort. Now this is a new vista in toxicology, and I think we have to face up

to something like you suggested here in order that decisions can be made during flight. These are ones where we get into what Maj. Reynolds has been talking about, where performance is critical. It is not a live or die situation. The really critical decision is can we detect and raise a flag early enough so that scrubbers can be changed, the cabin can be purged, or new techniques or other things undertaken before the mission is in jeopardy? This is a responsibility that the toxicologist must now evolve in the criteria of equal importance of what is toxic and what isn't.

DR. HARRIS: I want to thank Col. White for presenting my paper for tomorrow.

DR. STOKINGER: We will have to break now for coffee. Thank you.

Whereupon, the Conference recessed for a coffee break.

DR. STOKINGER: Will the meeting come to order. This is the period when we are going to have the panelists attempt to answer the written questions that have been so generously given to the podium here. We have about 50 of these questions that I am sure we can't answer in toto, so we will have to make selections from them, and I have tried to do this as we have been talking. We will start out with one. Mr. Rowe can answer this, and it is:- "For short-term space flight missions, from 7 to 30 days, materials must be chosen based on very limited data. What is your view of 1) selecting a screening material on the basis of odor tests, or 2) selecting a screening material on the basis of quantitative gassing tests, which identify the contaminants evolving from a material during a fixed period and which are then compared with the TLV -- these, divided by three, to compensate roughly for 24-hour exposures?" Do you want to direct your attention to this odor problem? -- Detecting odor and screening it on these space flights?

MR. ROWE: I don't think odor has much to do with toxicity but it might have to do with the person's acceptance of his environment. That's about as far as I'm going to go.

DR. THOMAS: I think tomorrow's session will take care of this.

DR. STOKINGER: Maybe we would like to have this question repeated tomorrow.

We had a question here directed to Dr. Reynolds:- "Soviet scientists divide animals into mentally sluggish and mentally alert, with corresponding toxicological inertness and responsiveness, and have applied this to man. Please comment."

MAJ. REYNOLDS: I think, of course, such measures are very crude by available techniques, and we know that the Soviets have used a number of

psychological "judgments". They have also relied to a great extent upon Pavlovian work for classically conditioned responses rather than on the operant techniques that are often used in the United States. First of all, I would say to the questioner that I must assume that this is a dichotomy, "mentally sluggish" and "mentally alert"; and, based on this, I think that the best way that the Soviets could approach it, if they are applying it to man -- the only way that I can see that it would be useful for them to approach it -- is to develop a scaling technique. This may be what they are trying to do, to go from a high level of alertness or sluggishness down to a very low level of alertness or sluggishness. The way that we have -- at least in the United States -- for handling these types of things with any reliability, is to develop a scale, say from one to ten, ten being the maximum level of alertness; and then have several trained observers who are observing what is going on on the part of the person or animal, correlate their observations. Then if you have, oh, five to seven observers, you can use something like Kendall's Coefficient of Concordance to see if the ratings are related to a significant degree. When your observers have been trained and can reliably agree on a given level of alertness, then they can make independent observations. From time to time you can bring them back together to evaluate their continuing agreement. So the only thing that I can say is that if the Soviets are applying such terminology to describe man's behavior, then the thing that they must do for it to be maximally useful, I think, is to scale these levels of "alertness" or "sluggishness" and to make sure that they have reliability among their observers. But I do think that this is crude by the techniques that are available to them, as well as us. And they are certainly available to them, as we know.

DR. STOKINGER: Any comment from the floor on this particular question? If not, we will pass on to another question, directed to Dr. Thomas. It says:- "What do you mean when you refer to chemical stress? Are you referring to pituitary-adrenal response? What plasma enzymes are you planning to measure as an index of stress and why?" Do we have time to answer all this?

DR. THOMAS: We refer to chemical stresses as some of the stresses which an astronaut in space travel will be subjected to, and we like to further differentiate into specific chemical stress with a specific response to a specific chemical. We feel that when you come to trace contaminants, very complex low level mixtures, you will not find a typical chemical insult -- this we call "nonspecific" chemical stress. We have seen this in our first continuous exposure studies at low concentrations, just like Capt. Siegel's studies; these were done at Midwest Research Institute, and as Dr. Back pointed out, there was no single common cause of death among these animals. What do we measure to quantitate stress? We would like to measure circulating epinephrine and norepinephrine. Our one big problem is the size of the blood sample required for these determinations. We are working on it. We are trying to reduce the quantity of blood needed. We are down now to where we can do it

with 5 cc plasma, but even this amount requires the use of large animals. With respect to which enzymes shall be measured, I should like to hear from the audience whether anyone has some suggestions on that, because I haven't made up my mind yet. Any suggestions?

DR. STOKINGER: Anybody from the audience that wants to propose some new enzymes that might be of value? I might suggest one for liver function tests that is coming to the fore now, and that is the ornithine carbamyl transferase. This is, allegedly, superior to the other types of liver function tests that have been in vogue for many years. We have not done enough in our laboratory to validate this item, but we are working on it, and we assume that it will hold up according to the merits that are proposed for it. Are there any other comments on this? Any other comments on enzymes?

DR. MURPHY: (from the floor - Harvard) I asked the question, I'll admit this. There are several enzymes that do respond to stressful stimuli. So my question, when Dr. Back indicated that they were going to do more blood enzyme studies to evaluate stress, was that I wondered what enzymes he had in mind. I don't know of any plasma enzymes, but certainly many liver enzymes are stimulated by glucocorticoids from hypersecretion of the adrenal cortex. Many of these can be measured quite easily but, of course, this means terminal sacrifice. I wonder if it wouldn't be worthwhile to include something like this as part of the study.

DR. HARRIS: This question was related to blood enzymes specifically; however, there has been some correlation between stress and urinary pepsinogen and urokinase. These might be well worthwhile including. They do not involve the matter of having to draw blood or terminal sacrifice.

DR. BACK: We are somewhat opposed to using enzymes just for enzyme's sake. You know there are dozens of these things, and they all have some activity certainly; but a reflection in blood enzymes is a rough road to hoe, especially in low dose. Capt. Siegel says he never finds anything in these enzyme studies, and we don't either; but, even if the animal dies, the enzymes appear still to be good. Therefore, I think that we are going to have to do something like Dr. Coulston is doing. We are going to have to do some punch biopsies along the way and do tissue enzymes. I think you remember not too long ago that we gave you an urgent call on the telephone saying, "what kind of an enzyme would you use for ozone", and you said, "glutathione and alkaline phosphatase", and we said "fine, send us your method". You did, and you told us it was serum glutathione and serum alkaline phosphatase. We ran both of them and didn't find anything. We should have done our homework but we didn't. Then we came to find out that sure, there were changes, as Dr. Murphy has shown many times, but at the tissue enzyme level. The tissue changes occur very early but are not reflected in the serum; so, probably we are at a standstill on serum enzymes. We are going to have to go to tissue. That's the only place where you are going to see a change.

DR. STOKINGER: This has pretty well been borne out by the work that Herb Cornish has been doing at the University of Michigan. He has been applying these serum enzymes to the leakage of enzymes from the liver in carbon tetrachloride poisoning, and as he exposed these animals to gradually decreasing amounts of carbon tetrachloride, one after another the enzyme activities that were inhibited, disappeared, and they were all normal when they got down to the levels that were representative of pretty high exposures. Is there any other comment on this question?

I have one here that probably Dr. Hodge would like to deal with. It says:- "The Conference has thus far placed great emphasis on the use of batteries of animal species, clinical observations and exposure conditions, in an effort not to miss anything in long-term exposure. No mention, however, has been made of the utility of preliminary short-term higher dose, any route studies, for an intelligent search for mechanism of action type of response to guide the scientific selection of our experimental procedures. Please comment on the need, or lack of it, for this type of study?"

DR. HODGE: Well, I'm all for science. If there is any way that we can approach toxicology more intelligently and with greater skill, certainly this is what we need -- not a standard screen on which you can shake a compound to see if it falls through. Many of the things said this morning have underlined the idea that a basic pattern of a few tests included in any inhalation study would permit cross-comparisons from laboratory to laboratory, but not at the cost of ruling out a true research approach to any compound. We just don't have enough time to do a standard screening study and then take off on what the real nature of the toxic effect is. We have to hit at the nature of the toxic effect from the word "go". The definition of research -- using your mind to its damndest, no holds barred -- describes the way we ought to approach every single toxicity evaluation. I don't think there has been any feeling that we ought to discard or discount the values that can come from one week or two weeks of preliminary exposure. I would like to commend the approach suggested yesterday, applying "Sydenham medicine" to toxicology, in which you watch the individual animal and its responses.

DR. STOKINGER: Is there any further comment on this question? I think that Dr. DuBois has a question or two that were given to him for answer.

DR. DU BOIS: I have been asked whether there have been physiological studies at altitude; first in relation to just atmosphere alone; and, secondly, toxic agents. In man, I think. Isn't that right? The question is on man?

DR. THOMAS: On man.

DR. DU BOIS: The ones I know about were evaluations in a space cabin atmosphere. They were run at Brooks Air Force Base. They were run for Republic Aviation by Helvey and others, Aircrew Equipment Laboratories,

first by Captain Gell, who was Director. More recently, there is one going on for 30 days for the so-called Apollo atmosphere, which I gather has been changed. The tests done were on the lungs in man and include vital capacity, maximum breathing capacity and chest X-ray at altitude; arterial blood oxygen and $p\text{CO}_2$ and PH at altitude; anemia, reticulocytes, blood and urine collection at altitude; peripheral vision. There were unanticipated effects of fire in at least two of these laboratories, and one might consider in this session what the effect of a fire might be on the environment and on the toxic products in this environment. There are other university laboratories that are continually studying physiological effects at altitude; among these, the University of Pennsylvania. Lambertson has a continuing interest in this. Rahn in Buffalo. Now I think there are fewer studies on toxic agents at altitude, I don't know of studies on that. Problems came up during the Gemini evaluation as to whether there had been contaminants in the atmosphere causing nephritis and casts in the urine, anemia with a reticulocytosis, and causing a change in peripheral vision. This underlines one basic difficulty of exposure, and that is, how do you know what is in the atmosphere to which the people are exposed in an altitude chamber? You can't analyze the atmosphere for trace substances of all sorts. Supposing you run mass spectrometer, it is not sensitive enough to pick up everything. You have to arrange gas chromatograph to detect certain substances of low concentrations. Things like Mercury, you have to have special detectors during the run so that you can look at the physiological effects and then afterwards you try to figure out what they were caused by and go back to the atmosphere and see if you can try to locate it. These were some of the problems that came up.

DR. THOMAS: We will hear a paper on this tomorrow from Mr. Conkle. They are analyzing one of their simulators continuously and freezing out various fractions of contaminants in traps and so forth. But I still cannot find anything published on measurements of pulmonary function at altitude. I am aware of the work that is in progress now, but those older papers you referred to measured their subjects prior to going to altitude, stayed up there for 30 days and, when they came back, measured them again.

DR. DU BOIS: In some of these tests, they've had respirometers in the chamber and were taking X-rays in the chambers.

DR. THOMAS: I'm talking about pulmonary diffusion function tests, like CO uptake, ethyl ether uptake, this kind of information. This comes the closest to the uptake of toxic agents.

DR. DU BOIS: At the current run at ACEL there will be some diffusing capacity tests but I don't believe those are going to be done in the chamber, because CO would contaminate the gas in the chamber; but gross mechanical tests have been done.

DR. THOMAS: There were no serious changes?

DR. DU BOIS: In the last run at ACEL a year ago, four out of the seven subjects developed atelectasis of the lungs. This was reversible but it was reproducible and occurred on several occasions.

DR. STOKINGER: I had a question closely related to what we were talking about. It ran something like this: What is the effect of 5 PSI 100 per cent oxygen on pulmonary diffusion? Can you make a general statement about this?

DR. DU BOIS:

- I. Diffusing capacity for CO at high altitude has been measured by Dr. John B. West, who found no difference from sea level, except for the increased reaction of CO with hemoglobin attributable to the hypoxia.

Ref: J. B. West. Diffusing capacity of the lung for carbon monoxide at high altitude. Journal of Applied Physiol. 17: 3, 421, 1962.

- II. Diffusing capacity at increased barometric pressure has been measured, and shows no change at 4 atmospheres.

Ref: Nairn, J. R., Power, G. G., Hyde, R. W., Forster, R. E., Lambertsen, C. J., and Dickson, J. The measurement of the apparent diffusing capacity for carbon monoxide (D_{LCO}) at hyperbaric pressures.

Abstract, The Physiologist, Vol. 7, No. 3, P. 211, August, 1964.

There are several different places where diffusion occurs. First, there is a diffusion into the bronchial wall and a reaction there of very soluble reactive gases, such as SO₂ and ammonia; these, where they are inhaled, tend to go into the surface mucosa in the tracheal bronchi and cause a reaction of bronchitis and bronchospasm. The ones that are less soluble or reactive with water go down further. These would be nitrogen dioxide and gases such as phosgene, which usually affect alveolar membranes. The next step in lungs involves a rapid diffusion of gas in gas from the lobule into the alveoli. This is a very short distance and diffusion occurs in an immeasurably short time. It's very short for all gases. Then through the alveoli membrane, it is a very short distance but it is watery. It has some lipid in it. Then the gas gets into the blood cells where it diffuses and reacts. Now with altitude, the diffusion of gas through gas would be a little quicker -- but it is so fast anyway that it is not a limiting factor, and so there would be no increased absorption of gases due to that.

Diffusion through the pulmonary membrane into the red cells wouldn't be changed, except that hemoglobin when it is less well oxygenated may take up carbon monoxide faster. The diffusion of particles of aerosols

from the lobule to the peripheral alveoli probably takes place more slowly, in a measurable time, and so at altitude it is conceivable that particles might diffuse more rapidly in the peripheral alveoli because the gas density is less. This remains to be seen. I don't know of anyone who has been testing this. The idea is that if you take in a breath of aerosol, you breathe out the same cloud of aerosol -- Dr. Norton Nelson showed this -- it hasn't actually diffused into the peripheral air spaces; except that with time it will, but this is for particle sizes between 1 to 5 micra. Gases behave as if they diffuse instantaneously.

DR. STOKINGER: Any comments from the floor on this, or questions?

"Can the panel elaborate on the increased sensitivity to a toxicant after exposure to the same toxicant?" There is another related question: "Is there any evidence of crossover effects; that is, is there evidence that some materials enhance sensitivity to a toxicant subsequently administered, as opposed to a synergistic effect?" Do you want to handle that, Harry? It's a hard one.

DR. HAYS: I don't know of any toxicant in the sense that we are talking about, that is, toxicants in the atmosphere, that increases the sensitivity to the toxicant after exposure to the same material. The only related study I can think of is the work of Dr. Amdur on SO_2 and aerosols where one gets a greater increase in the response to SO_2 in the presence of sodium chloride.

Is there any evidence of any crossover effect and is there evidence of some material that enhances sensitivity to toxicants subsequently administered?

I think that the classical example would be the increase in sensitivity to malathion by pretreating the animal with triortho cresyl phosphate. The LD-50 is 1200 milligrams per kilogram, and if you pretreat the animal with 3 milligrams per kilogram of TOCP, and then determine the LD-50, it is now 8 milligrams per kilogram.

DR. CARSON: I think I can add something to this kind of question. We have done a fair amount of work with a mucus flow technique using pulmonary irritants. The results are often surprising because we have been working with some of the ingredients in gas phase of tobacco smoke. If one deals with each of these individually and does studies so that we get resistance, compliance data, tidal volume, respiratory rate; and also puts this into a system in which we evaluate the changes on mucus flow in a living cat; then takes the various individual ingredients and puts them through a gas mixture, puts them all together, the unfortunate thing that I have to say is that the gas phase does not add up to one hundred per cent. So we are dealing with a series of physiological consequences of inhibition and a whole host of reflexes that get triggered during these exposure situations, and it behooves us then

to take a very careful look at individual ingredients and put them together very slowly if this is the intent of a synergism study, because occasionally inhibitory factors play a very strong role here.

DR. STOKINGER: Are there any other comments on this question? If not, we will proceed on to one that I think Dr. Thomas can answer. It says:- "Is there serious consideration being given to the establishment of a continuous toxic exposure criteria committee, similar to the ACGIH Threshold Limit Committee to publish maximal allowable continuous exposure levels?"

DR. THOMAS: I don't know of any. I don't think the state of the art is here in this area as far as research data goes. I think that in our space cabins, the contaminant picture will entirely depend on the type we use. I think Capt. Siegel would agree that every time you just change a piece of life support equipment, the contaminant picture will change. You will have to deal with them as they come up. I don't think there is any point in setting up any type of limit. The Air Force will give engineering criteria for our own systems, and NASA will do the same for theirs.

DR. STOKINGER: I think that is a good decision, because if you set a threshold limit, you set it on a single substance and not on a mixture that may be constantly changing.

CAPT. SIEGEL: I think it would be rather dangerous to set limits for either submarine levels or space levels and put them in the open literature. It is amazing how quickly things that are established for design criteria are misinterpreted as legal values -- values that you don't dare go one point above or one point below. So these are guidelines that we are thinking of, rather than any formal committee promulgation of standards.

DR. STOKINGER: In connection with your reply, Dr. Thomas, here is another question for you. It says:- "The results of continuous inhalation studies are of great importance to the non-military scientific community. What effort is being made to insure early publication of your results?"

DR. THOMAS: We publish our results, whether inhouse or contractor results, fairly currently; as soon as the final report comes in it is reviewed and edited, and the process takes from three to six months. These reports are available to the scientific public. They are available to our contractors without charge and to the public through Technical Services in Washington. If the work is of acute importance, such as I believe all this work on contaminant exposure is, I am certain that we will not only publish the report as an Air Force Technical Documentary Report, but will also allow it to be published in the open literature, unless for classified reasons it cannot be. So far, this area is completely unclassified. I have never yet run into classified compounds in space cabins. I hope it stays that way.

CAPT. SIEGEL: I have a comment. We are very slow in publishing, but all of our information is available to everybody here. Many of the people here have contacted us directly and we very willingly give the raw data. We are about three years behind in publishing because it is a one-man job and we can't get to it.

DR. THOMAS: By the way, the proceedings of this Conference will also be published as a technical report, and every attendee will get a copy. I also suggest that those of you who are deeply involved in this work send us a letter requesting that you be put on our regular mailing list.

DR. STOKINGER: We have time for just one more before the summation, and I think we might direct this to the Hodge-Rowe combination here. "Is this over-emphasis on standardization of methods permitting comparison between labs likely to stifle the imagination of investigators and thus result in the sterility of new developments and new basic information?" Does anyone want to handle that?

DR. THOMAS: May I attempt to answer this? We very carefully avoided using the word "standardization". This was not our intent. We said, let us set minimum criteria beyond which you are always welcome to go. If you find something better, we certainly want you to do it. But we didn't want to "standardize" procedures.

DR. STOKINGER: Do you have anything to say on that?

MR. ROWE: I think we answered that already this morning. I don't see any sense in going back to it. Do you know of something that we overlooked?

DR. STOKINGER: I think it was stated pretty well: Standardize the routine and leave the new special developments for the imagination.

I think this concludes the time that we have for this.

DR. HAYS: May I just say one more word in regard to standardization: It seems to me every time this subject has been raised, everyone has great fears that we are going to discourage basic research when we recommend standard procedures. I really see nothing terribly wrong in standardization. Those of you who are familiar with the development of pharmaceutical agents over the last 50 years will remember the U. S. Pharmacopoeia Committee and its contribution in developing standard procedures, which led to a better and basic understanding of the materials being administered to man. Many good research papers came out of this work. Digitalis would never have reached the stage of development if we all hadn't agreed many years ago that there had to be a certain degree of standardization. Every laboratory in the country was getting different results with digitalis and so a committee was

formed to set up some standard methods and standard criteria. Many research papers have come out of control laboratories where they are doing precisely the same fixed procedures day to day, but each assay, each control, is looked at from the point of view of basic research. I think it is a question of how you look at it. You need not be stifled by following some standard procedure.

DR. STOKINGER: Thank you, Harry. I'm sorry to say that we have more questions here unanswered on my desk than were answered. I've got nearly five dozen questions, and maybe tomorrow there will be some time in the procedure to answer some of these; because, as we found out, many of them were directed toward tomorrow's program anyway. So we will have to conclude now and listen to the resumé of these proceedings so far. Dr. Back will take this onerous task.

DR. BACK: We have heard over the past two days from individual papers, the panel and from audience participation, what should be done in space cabin toxicology. We have heard about how we should sample, how many animals we need, what species they should be and whether we need continuous or discontinuous exposures. I am going to try to indicate what we are planning to do and try to correlate all this information with what was said over the past couple of days. Our facility is going to perform continuous exposure experiments. That means that we are going to fly at altitude and stay there. We do not plan to bring our animals back to ambient pressures for clinical laboratory or other tests. Even though it is costly in terms of equipment, we are not going to take them out for any kind of manipulation. We plan to leave them right there, and anything that is done will be done at altitude. We plan to run almost every clinical test that Dr. Reeves talked about yesterday. We don't think that we can profitably do 90-day continuous work without obtaining every bit of data that the animal has within him. We do not plan to obtain all possible data from every animal. We will work on enough individual animals to prove our data valid. We plan to perform intensive work around one or more animals, as Dr. Carson and Dr. Hodge were advocating. So, to reiterate, we plan to stay at altitude, we plan to obtain every parameter that we can, within the realms of possibility, and we are going to use the macaca mulatta monkey. We don't plan to use the rabbit, except in rare cases. We plan to use rats and mice and at least three species of animals. We plan to run some 30 different clinical chemistries on the animals as necessary. Baselines will be obtained for about three to five weeks before we place them in the chambers for study at altitude. We are going to obtain data while they are there, especially the long-term studies, and then gather all the data we can when they come down. We plan to standardize every one of the clinical chemistries, as Mrs. Pinkerton said. We intend to use one method and one method only for each individual piece of data that we pick up. We don't have the time, money or the personnel to perform 30 different kinds of chemistry using duplicate or triplicate procedures, so we plan to pick one method and live with it. This is extremely expensive research and we just have to make a judgment concerning

the parameters to be studied, based on the contaminant, and carry it on. We will use macaca mulatta monkeys for a number of reasons. Yes, they have lung mites, and most seem to have them. We went through some 50 or 60 monkeys two years ago and X-rayed every one of them to find only 10 that didn't have a spot of some sort on their lungs. There just aren't many monkeys with clean lungs at this moment in the country. Dr. Reynolds indicated that we are going to do performance testing, and I think we are all coming to a point where toxicity isn't really what we are finally interested in; it's not toxicity that we are looking for, but the absence of toxicity. We are searching for a contaminant level in which an animal can perform, and we finally must come to a point where we find a dose level in which the animal can perform if he is motivated. Ultimately, we must take all our clinical chemistry, all our physiological work, all of our detailed pharmacological and toxicological parameters, and correlate them with performance; because, ultimately, we wish to know if the animal can adapt to his environment under these conditions. At the moment, we are still doing almost LC-50 type work in much of our toxicological endeavor, and we hope that psychopharmacological testing will add greatly to our knowledge. We are still working in an unknown area. We don't know what effect altitude has on toxicological changes and we are finally learning about this. We don't know about the effects of 100% oxygen. As soon as we know that we can work in 100% oxygen at 5 psi, we plan to go into details of how long and why we can work there. So we are still sitting on the ground, in this sense. Col. White came up with the thing that we are really interested in and that is performance. At this moment in time, one can't worry too much about long-term chronic effects. There is enough to do just to get off a relatively short mission. I should imagine that we are going to take a certain amount of risk no matter what we do. We at this moment can't predict whether one will acquire a chronic liver disease from our present flights. We must know, however, if an astronaut can perform. Can he perform his tasks at altitude and 100% oxygen for long periods? The only way we are going to learn is to have someone work in this environment. I think this is where we are finally going to put most of our eggs. The engineers are going to have to say, O.K., if we can't work in this atmosphere, how can we clean it up? They may clean it up to the extent where there is a tradeoff, or to the extent that the man can work. This may be the same point where the monkey can perform. In this case one takes a calculated risk if the rewards are high enough.

The clinical chemistries that we have seen haven't given us too many answers, and sometimes the gross and microscopic pathology doesn't give us the total answer. We must use performance, ultimately. So our next step is to work animals at altitude. We will obtain clinical chemistries, but it has been our past experience with certain compounds that performance alters before any blood or urine changes are evident. We've found out from our performance testing over the past 4 years that one of the first things that an animal does when he gets sick is to stop working. This is a good end point at which to stop the altitude experiments. If it is at the end of 60 days, O.K., we should stop the experiment after 60 days. We shouldn't have to go any

further unless looking for ultimate pathological changes. If the animal gets sick after five days of work, that's our end point. Our end point then isn't liver disease, kidney disease, or CNS pathology, per se -- but rather when an animal fails to perform his daily task. That's when we should stop because we've hit an end point. Of course, while we are getting these data, we must get the rest of it. If we don't, we can expect criticism, because our scientific colleagues will say, oh, you should have done this, or you should have taken blood serotonin, or you should have done fecal work, or you should have done this, that and the other, and this would be perfectly right. One should always obtain all the data he can from an experiment. So I think that a good end point for this work is performance.

A word about methods or the kinds of compounds that we are going to use and how to go about this, because our next big mission may be MOL. As you know, the other one is already locked in. How do we go about testing the many cabin materials which may be used? Our first effort is going to be to throw the lot in the chamber. If we put some of everything that is going to go in our space cabin in our domes and test it for 60 to 90 days, we ought to be in the ballpark as to whether the animals can live in that environment. If we throw everything in there and they get along fine, we've got it made. On the other hand, if we find that they don't live very long, then we've got to start pulling the atmosphere apart and treat each compound individually. However, we need the answers now, not 10 years from now, so one can't do the work on one compound at a time -- it's impossible -- there are too many materials. So we must put them all together and see what happens. If we are lucky, maybe we have no problems -- no toxicology problems. If we are not lucky, then we must do detailed work, the scutt work. This is very tedious and expensive; however, I think this is the path we have to take.

Now, as to the amount of animals -- some people have said two, some a dozen. We plan to use four to eight monkeys, ten dogs (both male and female), fifty rats, one hundred mice, and maybe some guinea pigs if we have room. In our long-term, one-year toxicity on oxygen, we will use twelve trained monkeys.

This resumé gets us where we are and where we in the Air Force hope to go. I would hope that others who get into this type of work will profit from our successes and failures in this tremendously interesting research effort. Thank you.

SESSION IV

TOUR OF INHALATION TOXICOLOGY FACILITIES AT AEROSPACE MEDICAL RESEARCH LABORATORIES, WRIGHT-PATTERSON AIR FORCE BASE, OHIO

The first portion of Session IV consisted of a conducted tour for all Conference attendees of the inhalation toxicology facilities of the Aerospace Medical Research Laboratories at Wright-Patterson Air Force Base, Ohio. The actual operation of both the ambient and altitude exposure chambers (Thomas Domes) was witnessed, and ample time was provided for specific technical questions and answers. The second portion of Session IV consisted of a formal banquet at which Mr. Pope A. Lawrence was the banquet speaker.

THE FEDERAL CLEAN AIR ACT
FOR
DOWN-TO-EARTH POLLUTION CONTROL IN UNCONFINED SPACES

By

Pope A. Lawrence

Chief, Federal Agencies Section, Abatement Branch
Division of Air Pollution
Public Health Service
Department of Health, Education and Welfare
Washington, D. C.

I appreciate the opportunity to participate in this Conference on "Atmospheric Contamination in Confined Spaces". I have no doubt that much of the new knowledge and criteria you are developing to ensure the safety of air breathed by humans during their missions into outer space or under the oceans will have useful application toward improvement of the quality of air breathed continuously by the millions of earth-bound citizens whose combined resources make possible those important missions. It is, therefore, with pleasure that I am here to present for you some of the highlights of the current Federal air pollution control programs and to trace briefly the development of a dynamic National policy toward conservation of our planet's atmosphere.

Evidence of increasing Federal concern for air quality was contained in President Johnson's message to Congress on February 8 of this year when he said:

"Air pollution is no longer confined to isolated places. This generation has altered the composition of the atmosphere on a global scale through radioactive materials and a steady increase in carbon dioxide from the burning of fossil fuels. Entire regional airsheds, crop plant environments, and river basins are heavy with noxious materials. Motor vehicles and home heating plants, municipal dumps, and factories continually hurl pollutants into the air we breathe. Each day almost 50,000 tons of unpleasant, and sometimes poisonous sulfur dioxide are added to the atmosphere, and our automobiles produce almost 300,000 tons of other pollutants."

President Johnson went on to comment on the pollution of streams and the matter of daily disposal of half a billion pounds of solid wastes adding:

"Almost all these wastes and pollutions are carried on for the benefit of man. A prime National goal must be an environment that is pleasing to the senses and healthy to live in."

"The Federal Government is already doing much in this field. We have made significant progress. But more must be done."

Evidence that the National policy toward air pollution is indeed dynamic was provided when the President added:

"I am directing the heads of all agencies to improve measures to abate pollution caused by direct agency operation, contracts and cooperative agreements. Federal procurement practices must make sure that the Government equipment uses the most effective techniques for controlling pollution."

In order to appreciate the present National policy on air pollution control, I think it is appropriate to review briefly the development of the Federal interest in air pollution as reflected in the legislative history and the evolution of the Federal program in this field.

Despite several widely publicized episodes of illness and death due to air pollution -- in Belgium, for example, in 1930, in Donora, Pennsylvania in 1948, in London in 1952 -- an identifiable Federal air pollution control program was not established in this country until 1955. In that year the 84th Congress passed Public Law 159 which enabled the Public Health Service to "provide research and technical assistance relating to air pollution control". In preceding years the Federal Government had done some work in this field. The Bureau of Mines, for example, had conducted some studies of the nature and control of pollutants from fuel combustion, and under the broad authority of the Public Health Service Act, the Public Health Service had conducted certain studies and investigations -- in particular, the one relating to the 1948 air pollution episode in Donora, Pennsylvania.

However, in July, 1955, with the passage of the original Federal legislation relating exclusively to air pollution, the Federal Government, through the Public Health Service of the Department of Health, Education and Welfare, began to develop and conduct a comprehensive air pollution control program. Congressional policy toward air pollution control expressed ten years ago in Section 1 of the 1955 Act was:

"That in recognition of the dangers to the public health and welfare, injury to agricultural crops and livestock, damage to and deterioration of property, and hazards to the air and ground transportation from air pollution, it is hereby declared to be the policy of Congress to preserve and protect the primary responsibilities and rights of the States' and local governments, in controlling air pollution, to support and aid technical research, to devise and develop methods of abating such pollution, and to provide Federal technical services and financial aid to State and local government air pollution control agencies and other public

or private agencies and institutions in formulation and execution of their air pollution abatement research programs."

Although the language of this Act was completely replaced by the Clean Air Act of 1963, one fundamentally important policy determination still remains to govern Federal plans and activities in the area of air pollution control. That is that primary responsibility for the control of air pollution rests with State and local governments and that the chief objective of the Federal air pollution program is to provide leadership and assistance to State and local control programs throughout the country.

Evolution of the Federal Program

The program developed under authority of the 1955 air pollution legislation was focused primarily on research and technical assistance. The research grew to include a broad range of investigations of the nature, effects and control of air pollution; and the disciplines involved included all the necessary branches of physical and biological sciences. In shaping this program, it was felt that effective control would depend upon (1) greatly increased knowledge of the types and amounts of pollutants being discharged to the atmosphere, (2) better understanding of the meteorological and climatological factors that influence dispersion of pollutants in the atmosphere, (3) more sophisticated knowledge of the physical and biological effects of pollutants, especially in relatively low concentrations usually encountered in community air, (4) greater awareness of the importance of certain specific air pollution emitters, such as motor vehicles, and (5) improved information of the administrative, legal, social and economic factors involved in the control of air pollution.

Prior to 1960, the air pollution activities of the Public Health Service were divided between two existing programs, one centered on medically oriented research, and the other on physical sciences and engineering. In 1960 these two programs were combined into one organizational unit identified as the Division of Air Pollution in the Bureau of State Services of the Public Health Service.

Changes in the Federal air pollution control program legislative authority were few between 1955 and 1963. In 1959, a provision was added directing Federal agencies to observe good practice in controlling air pollution arising from Federal installations and to cooperate to this end with State and local air pollution control agencies as well as with the Department of Health, Education, and Welfare. In 1960 an amendment directed the Surgeon General to make a two-year study of the health effects of motor vehicle pollution and to report the findings to Congress. The Surgeon General's report described motor vehicle pollution as an aspect of the air pollution problem endangering human health and that it could be expected to intensify unless remedial action were taken. Congress accepted this as fact and, as a matter of policy,

incorporated into the Clean Air Act of 1963 provisions reflecting its determination to maintain surveillance on the problem and progress toward solution. Several pieces of legislation now before the present Congress indicate intensification of Congressional concern that efforts toward prompt solution of the motor vehicle problem be pressed vigorously.

Current Federal Program

Clean Air Act of 1963

With adoption of the Clean Air Act in December of 1963, Federal policy in the field of air pollution control evolved significantly. Quantitative and qualitative information accumulated over the previous eight years influenced reshaping of Federal policy with respect to the kind and extent of aid to be made available to official control programs. Although there was no change in the view that responsibility for control of air pollution rests primarily with State and local governments, the Federal Government became much better equipped to aid State and local control programs more effectively and to stimulate them to increased levels of activity.

The preamble of the Clean Air Act reveals this changed viewpoint and policy concerning the Federal role in air pollution control. It cites the fact that most of the population now lives in urban areas reaching across municipal, county and State lines of jurisdiction. It reiterates the fact that air pollution endangers human health and welfare, and it specifically refers to motor vehicles as a major contributor to the Nation's air pollution problems. Most important, however, as far as Federal policy is concerned, the preamble to the Act states that Federal financial assistance is essential for the development of programs to control air pollution.

The Clean Air Act, for the first time, authorizes Federal grants awarded directly to air pollution control agencies to aid them in initiating, developing or improving their programs. These funds, which are intended as stimulants to accelerated local activity, are made available only on a matching basis. Qualifying programs created to serve intermunicipal or interstate areas are entitled to receive three dollars of Federal money for every one dollar of State or local funds. Strictly local programs are entitled to receive two dollars for every one dollar of local money.

Since approximately \$4,000,000 in Federal funds for this purpose became available last September, the response was so prompt that by March of this year requests considerably in excess of that amount were received. So far 65 awards have been made and these awards represent stimulation to 15 State, 25 municipality and 25 intermunicipal air pollution control programs. Nationwide air pollution control efforts by State and local agencies will increase by at least 40% in the coming year due to this Federal stimulation.

The Clean Air Act also provides, for the first time, legal regulatory authority, on the Federal level, for the abatement of certain air pollution problems. This regulatory power is clearly intended to supplement the abatement powers of State and local governments, and it can be exercised in two types of situations:

First, with respect to an interstate problem in which air pollution arising in one State is alleged to endanger the health or welfare of persons in another State, the Secretary of Health, Education and Welfare may, on his own initiative or upon official request as specified in the Act, initiate formal proceedings for the abatement of the pollution as found to be necessary.

Second, with respect to a purely intrastate air pollution problem, the Secretary may invoke such formal abatement proceedings but only on official request from designated officials of the State involved.

The regulatory abatement procedures authorized in the Act are similar to those used for several years under provisions of the Water Pollution Control Act; this involves a course of action which can include consultation, conference with cognizant official agencies, public hearings and finally Federal Court action. The procedure may, of course, terminate at any step of the process if the problem is resolved.

Under the new abatement authority contained in the Clean Air Act, the Department of Health, Education and Welfare has initiated an abatement action in the New York City-Northern New Jersey metropolitan area. A consultation has been held with appropriate State officials concerning air pollution arising in New Jersey and alleged to endanger public health and welfare in parts of New York City. Following this meeting, technical data bearing on the problem were assembled and evaluated. The next move rests with the Secretary of Health, Education and Welfare.

The new and evolving Federal air pollution control policy is reflected in other provisions of the Clean Air Act. For example, the Act directs the Department of Health, Education and Welfare to develop and promulgate criteria for air quality for the guidance of State and local authorities in establishing standards for source emissions and ambient air.

Present work in this area is concentrated on the oxides of sulfur and photochemical oxidants such as ozone and PAN, but other pollutants are scheduled for intensive study leading to establishment of criteria.

Under a provision of the Clean Air Act relating specifically to air pollution generated by motor vehicles, the Secretary of Health, Education and Welfare recently reported to the Congress his conclusions relative to the need

for additional efforts to deal with it. The report concluded in part that a need exists for the development of means to insure both nationwide application and appropriate maintenance of available systems for the control of motor vehicle emissions. Legislation has already been introduced in the Congress to insure that new American-made and imported cars include exhaust and crankcase emission control systems.

The Clean Air Act has led to a number of actions by the Executive Branch, which in themselves both reflect and contribute to the formation of National policy on air pollution control. For example, the Executive Office of the President, through the Bureau of the Budget, is now in process of issuing instructions for Federal departments and agencies, directing them to cooperate with the Department of Health, Education and Welfare in planning air pollution control measures to be incorporated in new Federal installations.

Federal Agencies

I doubt that it is generally realized that the Federal Government owns in the United States over 15,000 installations including over 400,000 buildings, and that it leases space in over 40,000 buildings. Except for a few unique situations, Federal installations produce air pollution comparable to that associated with emissions from non-governmental sources in urban areas; e.g., smoke and fly ash from heating plants, power generating stations, incinerators and open fires. A few specialized problems involve disposal or salvage of large quantities of waste materials, such as harbor debris or obsolete military equipment and materials, and emissions from rockets and missiles.

Since inception of the first identifiable Federal air pollution program, the Public Health Service has held and advocated the policy that Federal agencies should be exemplary in preventing and controlling air pollution arising from government property. This view was first formalized in Executive Order No. 10779 in 1958. It gained strength by Congressional policy declarations in 1959 and in the Clean Air Act of 1963. The present Congress is considering new legislation intended to guarantee the accomplishment of this objective; and earlier in this talk I mentioned President Johnson's statement of February 8 on the subject. I think it particularly significant for this audience to recall that the President directed all agencies and departments to improve measures to abate air pollution caused not only by direct agency operations, but also operations generated by contracts and cooperative agreements.

I feel confident that this objective set for Federal agencies will be accomplished, especially in view of the Senate's action last Thursday (March 25) in unanimous passage of Senate Bill 560. The essence of this new legislation, which, of course, still requires action by the House, puts all Federal agencies on notice that Congress expects them to seek and use funds necessary for the control of air and water pollution.

The Public Health Service Division of Air Pollution has found that persons in charge of Federal facilities usually are cooperative and sensitive to their responsibilities as good neighbors in their communities. The major deterrent to corrective action in the past has been lack of adequate funds. However, last year the Bureau of the Budget took constructive action toward removal of that obstacle by amendment to its Circular A-11 requiring all Federal agencies to include provision for the control of air and water pollution at all new Federal facilities and buildings. These provisions are now being made in accordance with instructions prepared by the Public Health Service. It may be anticipated that additional instructions will be forthcoming for application to air pollution control at existing installations. Formulation of some of these new instructions will require the help of many of you in the scientific community represented at this Conference.

Conclusion

The new Federal program emerging from impetus of the Clean Air Act and subsequent actions of the Executive and Legislative Branches of our government constitutes a clear invitation to public and private interests to accept their responsibilities for air pollution control. The Federal Government is committed to a program of strong leadership and assistance to ensure that the task of getting air pollution controlled is indeed accomplished.

Air pollution can no longer be tolerated nor dismissed with excuses, half way measures, nor promises of future progress. Inhabitants of this planet cannot now afford to become so preoccupied and enthralled with the wonders of space, dehumanized technology and our marvelous capabilities for control of vast amounts of energy that we forget conservation of life on Earth. We must remember that organisms, ecological systems upon which we depend, human beings and not least cities themselves, are delicate devices for regulating energy and putting it to the service of life. The Public Health Service has always been motivated by that idea and this Conference reveals evidence that you share this idea, too.

Together, then, we can and I believe we will move ahead with our Nation's multifaceted business, keeping before us the goal that our environment shall, as the President said, "be pleasing to the senses and healthy to live in".

SESSION V

TOXICOLOGICAL QUALIFICATION OF SPACE CABIN MATERIALS

Chairman

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SESSION V

Opening Remarks

Dr. George Kitzes, Conference Chairman

During the Session III panel meeting we discussed the problems confronting us in the anticipation of the requirements of obtaining data from continuous exposure inhalation toxicology. It may appear strange to some of you that we should try to discuss the problem before the actual user, or one of the users, talked to us about what he has already found. In anticipation of space flights, we know that in confined volumes like space cabins and submarines, man cannot readily leave his environment should he have occasion to encounter intolerable odors, irritants or whatever you can expect in the way of air pollution. Certainly in Los Angeles, as was mentioned yesterday, if you don't care to go downtown and be exposed to the irritants you may stay home, or you may wish to go to the mountains for a breath of fresh air. We are not going into too much detail. We realize that this is the nature of the situation, the nature of our problems, and this is why we are here and have been here the past day and a half. We anticipate special problems because man will be confined to his space cabin or to the submarine. Captain Siegel of the Navy has similar problems and actually had commenced to study these a year or two before NASA and the Air Force had occasion to consider them for their aerospace systems. However, in terms of space cabins, we have an additional consideration; and that is, in many instances both NASA and the Air Force may have to use reduced ambient pressures. Here's where we have the gas-off problem. You might say that there is considerable variation in the gas-off properties of the materials that may appear in the cabin. Then again you may question the seriousness of this problem because of the extent of reduced ambient pressures. Certainly we are not considering these reductions with that of open space. We have to think in terms of the present 5 PSI, 7-1/2 PSI, and perhaps we may find that it's desirable to achieve what the Russians have done and go to the 12-1/2 to 15 PSI; but, meanwhile, there are more immediate problems in terms of the 5 PSI and the 7-1/2 PSI. We do have the gas-off problem. Consequently, we are concerned with the nature of these gas-off materials.

Moreover, in a confined volume like a space cabin or submarine, we're confronted with multiple items. I think this was well illustrated in the paper given by Dr. Hueter from the Public Health Service in considering the exhaust products of automobiles. I think he had already arrived at the point where he found it was not practical or feasible to consider each and every one of the materials that were identified in the exhaust, but treated them as a whole. This is something that we may have to do when we consider the contaminants.

For Session V we have a Chairman and a Co-Chairman because the problems associated with toxicological qualification of space cabin materials are tied in with biochemistry and toxicological criteria, and also engineering

criteria; because, essentially, all the data that are generated from the exposures, all the toxicological data, have to be reinterpreted or interpreted for the engineer's use. So, we have Dr. Harris from NASA with us as Chairman, and Mr. Roundy from the Aerospace Medical Research Laboratories as the engineer, as Co-Chairman.

I would like to mention that NASA is as equally interested in the problem as the Air Force, and has recognized the value of our chambers, the unique capability that we have at Wright-Patterson. We should have a coordinated program to make maximum use of our national facilities, and I should mention that much of the work that was presented in the past day and a half has been partially supported by NASA funds.

PROBLEMS OF SPACECRAFT MATERIALS SELECTION AND TOXICOLOGICAL EVALUATION

By

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INTRODUCTION

A large number of materials used in spacecraft construction are the product of a relatively new technology -- the production of plastics and elastomers having specific mechanical properties for specific uses. Many of these products, when exposed to the atmosphere, lose solvents, plasticizers, and unpolymerized materials by evaporation; and, at elevated temperatures, may even react with the atmosphere.

In an open environment these products are readily dissipated and only in exceptional cases are considered to be public health hazards.

In a closed environment, however, many of the gas-off products may accumulate, and thus constitute a toxic hazard.

CURRENT SPACECRAFT

At present, NASA's Manned Spacecraft Center has Gemini, the Apollo Command Module and the Lunar Excursion Module spacecraft to consider in terms of the inhalation toxicity which could possibly result from the off-gassing of non-metallic materials within the confines of the habitable area of the spacecraft.

These vehicles are well into the design and construction phases so that little can be done to alter the use of non-metallic materials within the habitable area of these craft. The maximum mission lengths for Gemini and for Apollo are 14 days with a maximum anticipated occupancy of the Lunar Excursion Module of 48 hours.

Criteria Used for Non-Metallic Materials Selection

The criteria used to ensure an innocuous atmosphere in the spacecraft have not been quite the same for each spacecraft manufacturer.

Gemini. - The manufacturer of Gemini (fig 1) determines the acceptability of a non-metallic material by establishing the maximum temperature at which it is anticipated that it will be used. The acceptance limits are based upon 3 to 24 hours of continuous use tests in 100 percent 5 psia oxygen without producing irritating or obnoxious odors. Toxicity and temperature limits, although stated together, are not based upon actual tests (ref 1).

Apollo. - The acceptability of non-metallic materials for the Apollo Command Module (fig 2) is based upon heating to 200°F in 100 percent oxygen at 5 psia for 48 hours. A gas sample is drawn off, passed through the gas chromatograph, and the total organic content of the vapors, as determined by a flame ionization detector, is expressed as pentane equivalents. Condensable materials are determined by evacuating the test chamber into a condensate trap and then returning non-condensables to the test chamber. The test chamber is brought to atmospheric pressure with oxygen and tested for odor by use of a Fair-Wells type olfactometer. The specimen weight change is determined (ref 2).

For a material to be considered acceptable, it must meet the following criteria:

1. The total weight loss, exclusive of H₂O, must be less than 1 percent.
2. The total organic off-gassing should not exceed 100 ppm by weight when expressed as pentane equivalents.
3. The material after heating must not have a mean odor exceeding 1.5 on the basis of an olfactometric test in which four panel members rate the odor as:

Not detectable	0
Detectable	1
Objectionable	2
Irritating	3
4. No observable room temperature condensate other than water is obtained.

Lunar Excursion Module. - The test program for the qualification of the non-metallic materials used in the habitable area of the Lunar Excursion Module (fig 3) is similar, in many ways, to that used for the Command Module (ref 3). The non-metallic materials are heated to 200°F for 72 hours in 100 percent 5 psia oxygen. The products that off-gas under these conditions are trapped, separated chromatographically, and identified by the combined or individual utilization of mass, ultraviolet and infrared spectroscopy.

Odor evaluation is performed by subjecting the material to the conditions described previously. The gasses are brought to atmospheric pressure with oxygen and the odor is evaluated by a panel of 10 male non-smokers.

The basis for elimination was similar to that used for the Command Module, but also included ratings for "pleasant" odors, since some odors which may be initially pleasant may, on prolonged inhalation, become sickening (ref 4).

Since the limit of continuous occupancy of the Lunar Excursion Module more closely resembles that upon which threshold limit values of toxic chemicals are based, the quantity of the most toxic substance off-gassed is taken as the basis for establishing a maximum limiting weight for the use of a given material. If a number of materials off-gas a common product, the total quantity of all these materials is limited by the maximum allowable concentration of the common substance off-gassed (ref 5).

Crew safety and performance is, of course, paramount in importance. Each of the methods for materials selection has its merits and, of course, its controversial aspects.

Factors Contributing to Crew Safety

An ideal solution for materials selection has not yet been presented. However, in terms of crew safety and performance, there are several factors which aid in minimizing the risk of a toxic response. First, the off-gassing substances should be low molecular weight additives and unpolymerized material with relatively high vapor pressures and thus should off-gas rapidly under reduced pressure. Second, the Apollo Command Module, the Lunar Excursion Module and Gemini have anticipated leak rates of approximately 0.2 lb/hr at 75°F and 5 psia pressure. This provides a slow, but effective, removal of contaminants from the atmosphere. Third, the presence of activated charcoal and LiOH in the environmental control system plus the rate of atmospheric turnover provides for the removal and adsorption of atmospheric contaminants. In Apollo, the gaseous turnover in the Environmental Control System amounts to 36 cfm of gas passing through the LiOH and activated charcoal bed. In Gemini it amounts to 22-23 cfm, and in the Lunar Excursion Module it is approximately 24 cfm, or approximately 12 cfm per man in each spacecraft. Fourth, isolation or removal of as many components as possible from the crew compartment effectively eliminates these materials as potential contaminants. Fifth, the space vehicle will be off-gassed during preflight decompression tests with all systems operational, thus reducing the quantity of material available for atmospheric contamination during flight.

These factors are not meant to imply that a contaminant buildup cannot occur, but, as a result of the factors enumerated previously, that probability is quite low.

Toxicity Testing of Off-Gassing Products

Since the 14-day Gemini mission, and the 14-day Command Module missions do not have any programmed extravehicular maneuvers which would

reduce the contamination levels, we are engaged in a program to determine the possible toxicity of groups of materials off-gassed on a unit-weight basis with maximized surface area. Groups of 10 or more spacecraft non-metallic materials are heated in 100 percent oxygen at 5 psia, and the off-gassing products cycled continuously through 20 liter chambers containing the test animals. In the event toxic responses such as death or behavioral changes are observed, the toxic materials will be identified by a cascading process of subdivision into groups of 5 and 5, 3 and 2, et cetera. Large initial groups would be too unwieldy to handle in the event of a toxic response. Smaller groups would prolong the testing unduly. In addition to the toxicity testing, the Manned Spacecraft Center's Systems Test Branch is trapping and quantitating off-gas products during Gemini Systems test runs to determine the types and quantities of gases produced under operating conditions.

FUTURE GENERATION SPACECRAFT MATERIALS REQUIREMENTS

As future generation spacecraft missions increase in length and as the spacecraft becomes tighter, that is, leakage decreases and possible decompressions become less likely, the more stringent should become the management program for materials selection, control and decontamination to avoid toxic hazards.

Approach to Materials Selection Program

I would like to present a possible approach to such a program.

Examination of materials catalogs, journals and current spacecraft materials lists indicates a vast number of materials having similar functions. Many of these materials are being used on current generation spacecraft, and off-gassing and toxicity information concerning them is being obtained.

So vast is this number of materials that an examination of the materials used in the current generation of spacecraft provided a list of over 300 materials belonging to 20 generic classes and generic combinations with only a dozen of these materials being used in more than one spacecraft.

Maximization of Redundancy

Thus, the first stage to consider in establishing future spacecraft material selection specifications is the maximization of redundancy of utilization during the design stage substituting only when continued use constitutes a hazard. This, in effect, should reduce, but not eliminate entirely, the requirements for toxicity testing beyond the design stage. The current lack of redundancy is an artificial one. It is caused by variations in nomenclature,

trade names and personal preferences and prejudices. To carry this point to extremes, one manufacturer listed the same material under two different names on the same spacecraft. Upon inquiry it was found that the supplier had changed the product's name. Therefore, the manufacturer carried both names on his materials list.

It becomes essential, therefore, that the number of different materials used in the spacecraft be reduced to an absolute minimum. NASA should be and is obtaining knowledge of off-gassing and toxicity of the materials used in current-generation spacecraft in collaboration with the Air Force Toxic Hazards Branch at the Wright-Patterson Air Force Base.

Standardization of Materials Selection

The methods for study of the off-gassing must be standardized and rapid screening procedures must be used to reduce the number of materials tested for long-term off-gassing to a bare minimum. This means that suppliers will have to comply with a set of space material specifications as they do with Military Specifications.

As the spacecraft design engineers progress, they will specify material requirements based upon use and structural properties.

The initial "source book" for such materials should be NASA generated data on the kinetics and toxicity of the off-gassing products of materials used in the current generation of spacecraft. Materials having all of the desired properties might not be found there. Therefore, by a process of cross-matching application requirements and generic groupings, an additional abbreviated list of materials possibly suitable for spacecraft use could be obtained.

Methods for the Testing of Non-Metallic Materials

The methods for the selection of the non-metallic materials from this list do not have to differ greatly from those currently in use for spacecraft materials qualification.

Odor Test. - The primary test would be an odor test similar to the one used by Grumman Aircraft Engineering Corporation, a variation of which is being considered for use in the selection of materials for all current spacecraft (ref 6).

The test panel consists of 5 to 10 men selected on the basis of their ability to differentiate between seven basic odors. The material, heated to 200°F for 72 hours is brought to atmospheric pressure with oxygen. The

sample is diluted and presented to the panelist by means of a syringe injected into a flexible plastic cup as shown in figure 4. The panelist records his estimation of the odor as follows:

Undetectable	0
Barely detectable	1
Easily detectable	2
Strong or irritating	4

The test coordinator assigns values of zero, one, two or four, respectively, to the evaluation. If the mean value of 10 or more determinations by the panelists exceeds 2, the material is rejected.

Those materials which fail this test may not be used in the spacecraft habitable area. Those materials which are considered acceptable on an odor basis would then proceed to the next test -- thermogravimetric analysis.

Thermogravimetric Analysis. - The parameters for acceptability based upon thermogravimetric analysis are currently under discussion. An ideal situation is shown as the top line of figure 5; that is, no change in weight with a programmed increase in temperature. An unacceptable material may show a continuous change in the rate of off-gassing in the temperature range explored (bottom line of fig 5). An acceptable material, however, would be a compromise, such as the center line of figure 5. For the purpose of simplicity the figure shows only a single off-gassing component.

The temperature profile for the rates of gas production and the rates at which the materials reduce their off-gassing properties can be determined from the slopes of the curve.

Those materials which fail the above tests may be either rejected, further studied to establish quantitative acceptability, treated to provide acceptable standards or relegated to an area outside the habitable portion of the craft.

Kinetic Studies of Off-Gassing and Contaminant Removal. - The next phase of the proposed program for those materials which have passed the odor and thermogravimetric tests is the determination of the kinetics of off-gassing under anticipated spacecraft conditions. The identity of the off-gassing products, the rate at which they off-gas as a function of material weight and surface area, and the anticipated drop off of out-gassing with time must be obtained.

The spacecraft design engineer should make use of this information for pre-treatment, coating or reduction of contaminants by a contaminant control system. This system may be as simple as an activated charcoal bed or a more complex one containing an activated charcoal bed and a catalytic

burner backed up by LiOH (ref 7). There are going to be materials whose off-gassing products cannot be completely removed by the contaminant control system. These materials must be tested for toxicity. Not all ills can be cured by pre-treatment, isolation or complete removal. For these conditions, mission simulation for toxicity would appear to be the answer.

Computer Application

NASA has developed a computer program which will handle the generic type of the material, its' trade name, its' properties, uses, weight in spacecraft, and the spacecraft or component in which it is used. With minor changes and off-gassing data provided, the computer can follow the buildup of noxious substances and their rates of accrual.

This program, as now set up, is strictly a data retrieval system; and, as such, is inadequate for mission simulation.

A computer system, however, may be the ultimate answer to the problem of spacecraft toxicity testing, but it will require the utmost cooperation among design, materials test and toxicity groups.

Mission Gas Profile. - Rather than use the computer as a strictly data retrieval system, it should be provided with the kinetics of off-gassing as a function of weight, surface area and time under nominal service conditions as obtained from the design specifications. It should be provided with the efficiency with which known contaminants are eliminated by the contaminant removal system of the vehicle, the experimentally determined rate of degradation of the contaminant removal system, and the rate of turnover of the spacecraft atmosphere through the contaminant control system. With this knowledge, the computer should be able to predict:

1. The rate of buildup of a specific contaminant
2. The peak value for a given contaminant and the time at which it will be reached
3. The eventual equilibrium value, if any, of a specific contaminant and the time required to achieve it

Toxicological Mission Simulation. - Using this information it should be possible to simulate a mission toxicologically, utilizing a programmed trace contaminant simulator such as is being developed for NASA by Honeywell. The model being developed will contain 10 channels, one for each contaminant. Each channel will have the capability to provide release rates of 10^{-6} moles of gas/hr to 10^{-2} moles of gas/hr (ref 8).

A combination of the computer-plotted mission gas profile, the controlled contaminant leak system and animal chambers will provide a practical

capability for determination of the toxicity of the spacecraft atmosphere prior to and during the course of its construction without having to resort to extensive toxicity testing of each material.

Implicit in this whole program is C O N T R O L. We cannot have, as has occurred in our off-gassing studies, one lot of material gas-off 2.1 mg of propanol in 30 days, whereas a similar quantity of a second lot of the same material off-gassed only 0.5 mg of propanol in 60 days.

The success or failure of any reasonable attempt at establishing a long-term habitable atmosphere without monopolizing the toxicological facilities of the nation rests upon a cascading function of selection and the establishment of meaningful specifications relating to methods of manufacture and allowable limits for volatile components.

To summarize, we have then the following functions to achieve mission objective, that is, a non-noxious atmosphere in the spacecraft habitable area:

1. Spacecraft design
2. Materials selection
3. Contaminant control
4. Toxicity evaluation by simulation
5. Spacecraft atmospheric validation

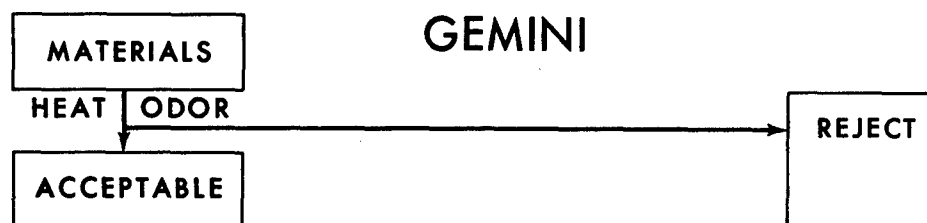


FIGURE 1.

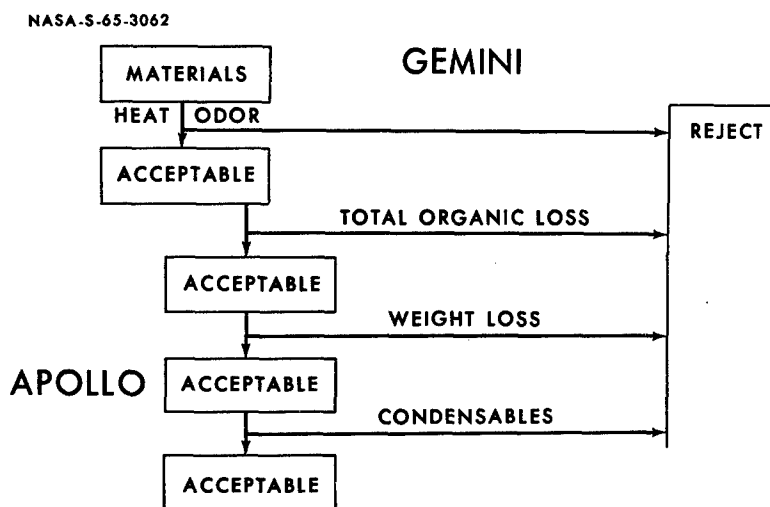


FIGURE 2.

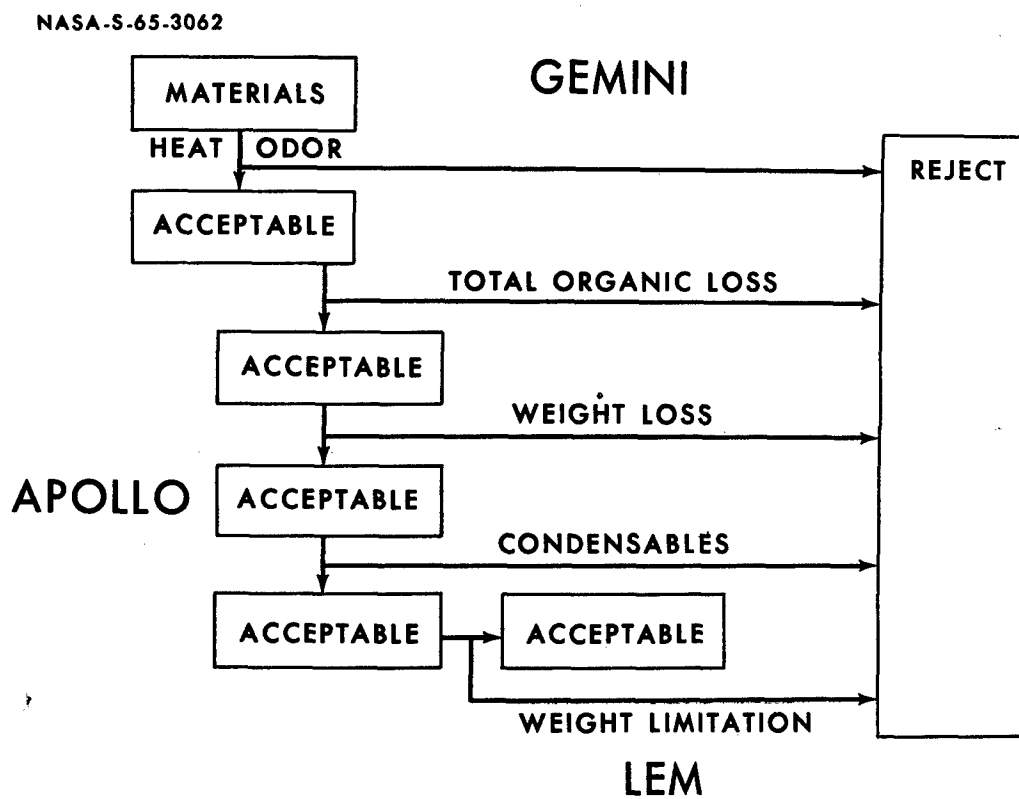


FIGURE 3.



FIGURE 4. GRUMMAN AIRCRAFT ENGINEERING CORPORATION - ODOR TEST

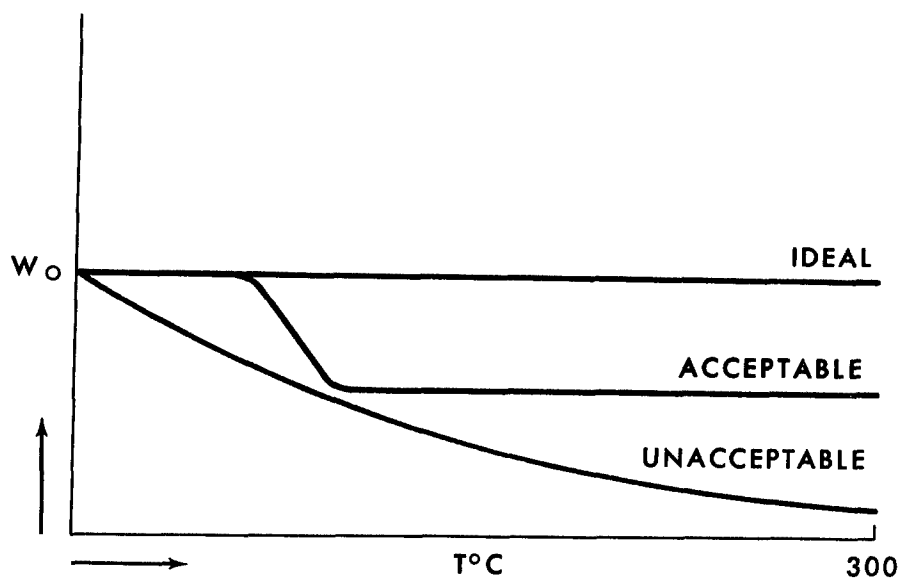


FIGURE 5. THERMOGRAVIMETRIC ANALYSIS

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LIFE SUPPORT EQUIPMENT AND ITS CONTRIBUTION TO CONTAMINANT GENERATION AND REMOVAL

By

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The life support equipment in a closed or partially closed environment has three primary functions; namely, maintenance of a habitable atmosphere, storage and dispensing of food and water, and storage and processing of waste products from man or other sources. The above functions must be performed with proper regard for ability to accomplish the assigned mission, safety, comfort and personal hygiene and sanitation. Implicit in the life support function and in man's relationship to the life support system is the requirement that the atmosphere, food and water provided to the man be as free from contamination as may be required for mission success. Establishment of permissible concentration levels for various atmospheric contaminants is of considerable importance to life support system designers since contaminant control devices can be built into the life support system. Proper selection of contaminant control techniques and devices, coupled with the knowledgeable selection of all materials within the closed environment, are major factors in the control of contaminant levels.

Many components or materials used in life support systems are similar to those of other systems proposed for use in closed environments and, although contamination problems may arise from these items (ref 1), they will not be discussed here. Components, materials and techniques unique to various life support systems and which may decrease or add to the contamination problem will be discussed.

The food and water storage and dispensing systems, while subject to internal contamination problems, are not considered likely to contribute to atmospheric contamination. However, if heated and/or chilled foods and water are to be provided, the equipment and materials of construction used in the heating and cooling devices must be selected with proper regard for prevention of atmospheric contamination.

The systems for storing and processing waste products involve two general areas. One is concerned with the management of solid waste, such as garbage, feces, used food containers and other debris. Sanitation and personal hygiene (ref 2) are involved and methods for the long term storage

or disposal of these waste products which preclude atmospheric contamination are being developed. The other area is concerned with the reclamation of potable or usable water from urine (wash water and condensed water from the atmosphere can be processed by techniques used for urine processing or by simpler methods). A large number of water recovery methods have been investigated (ref 3) and some of the equipment employed is rather complex. A number of systems operate at pressures below that existing in the closed environment (low temperature distillation, for example) and contamination of the atmosphere from the material being processed is unlikely in these cases. Pre- and post treatment of the material is required in some techniques and antifoaming agents, ion exchange resins, various oxidizing agents, etc., which may be employed, could contribute to atmospheric contamination.

That portion of the life support system which provides for the maintenance of a habitable atmosphere is directly involved in control of atmospheric contamination. Two general types of systems will be discussed and specific techniques and materials applicable to various portions of these systems will be pointed out. Both systems involve the continuous removal of gas from the closed environment, the processing of this gas, and the return of the usable portion of the gas for breathing purposes. The basic nature of these systems makes the introduction of a device (often a canister of activated charcoal) for the control of contaminants an easy matter. What the device should be, and indeed the detailed nature and amount of the contaminants it should remove, present very difficult problems. The first type of system (figure 1) involves the circulation of gas from the closed environment through components which remove carbon dioxide and water vapor. Water vapor is often removed by condensation on a cold surface although Molecular Sieve, silica gel, and other adsorbents can also be used. Carbon dioxide can be removed by adsorbents such as Molecular Sieve and activated charcoal or chemically by lithium hydroxide, Baralyme, etc. Systems using adsorbents often provide for cyclic operation of two adsorbent beds (one being reactivated for future use while the other is adsorbing). All of the above systems have been investigated and designs, which make contamination of the atmosphere from these systems highly unlikely, have been developed.

The second type of system (figure 2) can employ the same techniques for water vapor removal as the first type. It must, however, provide for removal of carbon dioxide and transfer of the carbon dioxide to a decomposition system (ref 4) where breathable oxygen is recovered. A reversible adsorbent system is applicable to the carbon dioxide removal component. The carbon dioxide decomposition apparatus could contribute to atmospheric contamination. Three particular techniques for carbon dioxide decomposition should be mentioned. The first involves the reduction of carbon dioxide with hydrogen to form methane and water with the water in turn being electrolyzed to release oxygen (hydrogen released in the electrolysis process is reused for further CO₂ reduction). The second utilizes hydrogen to reduce the CO₂ to

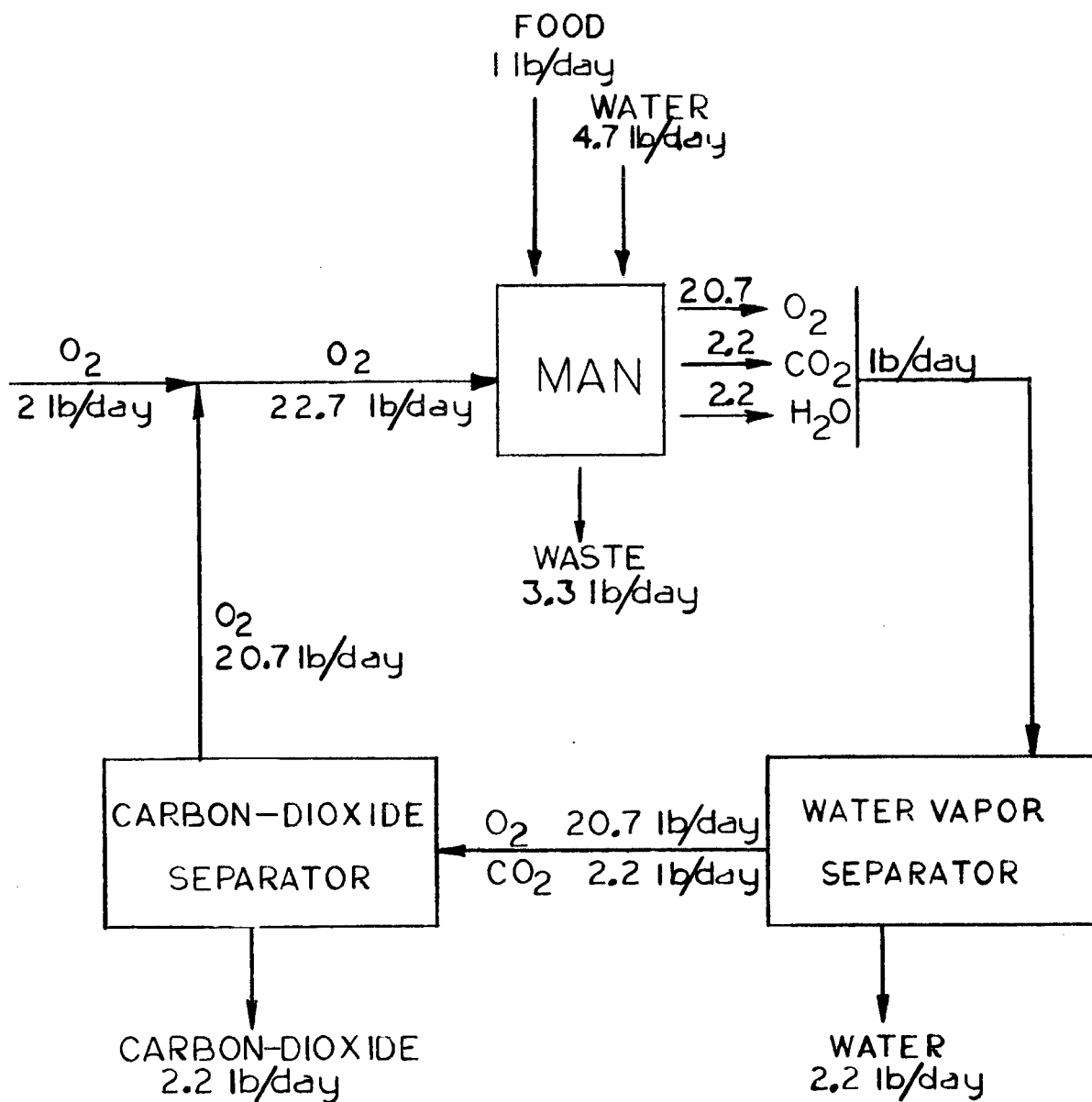
carbon and water (the water is electrolyzed as in the first process). Recycling of the processed gas is required and methane and carbon monoxide are produced in the system (ref 5). The third system employs a solid electrolyte of zirconium and yttrium oxides (ref 6) to decompose the CO_2 into carbon monoxide and oxygen. The carbon monoxide is further processed to form carbon and carbon dioxide which is returned to the electrolytic cell. In all of the above processes, the possibility of leakage of the various gases involved exists and must be considered in the equipment design.

The processing of the closed environment atmosphere in the above systems is aimed primarily at carbon dioxide and water vapor removal. The effect of the various chemicals and treatments on possible atmospheric contaminants in general has not been investigated to any great extent.

The leakage rate of atmospheric gas from a closed environment is of considerable importance to the life support system designer since the system must provide replacement gases (oxygen and a diluent, if used) for those lost due to leakage. Leakage, of course, aids in reducing the level of atmospheric contamination and, in some cases, can be used to control this level. Under steady state conditions, a simple relationship exists between the contaminant introduction or generation rate (I), the concentration of the contaminant in the closed environment (C) and the leak rate (L). This relationship is $I = C \times L$. The equation could be applied to the laboratory determination of contaminant gas-off rates from materials at reduced pressure. The leak rate for existing aerospace systems is usually predicted during design and later determined by actual test (one Project Mercury capsule had a leak rate of 0.03 kilograms per hour, for example). If the contaminant introduction rate were known for one of the above systems, the expected concentration level of the contaminant could be determined.

In summary, the general areas involving life support equipment have been defined and the techniques and materials used have been indicated. More emphasis has been given to the habitable atmosphere area because of its direct involvement in the contaminant removal process.

CLOSED RESPIRATORY SYSTEM

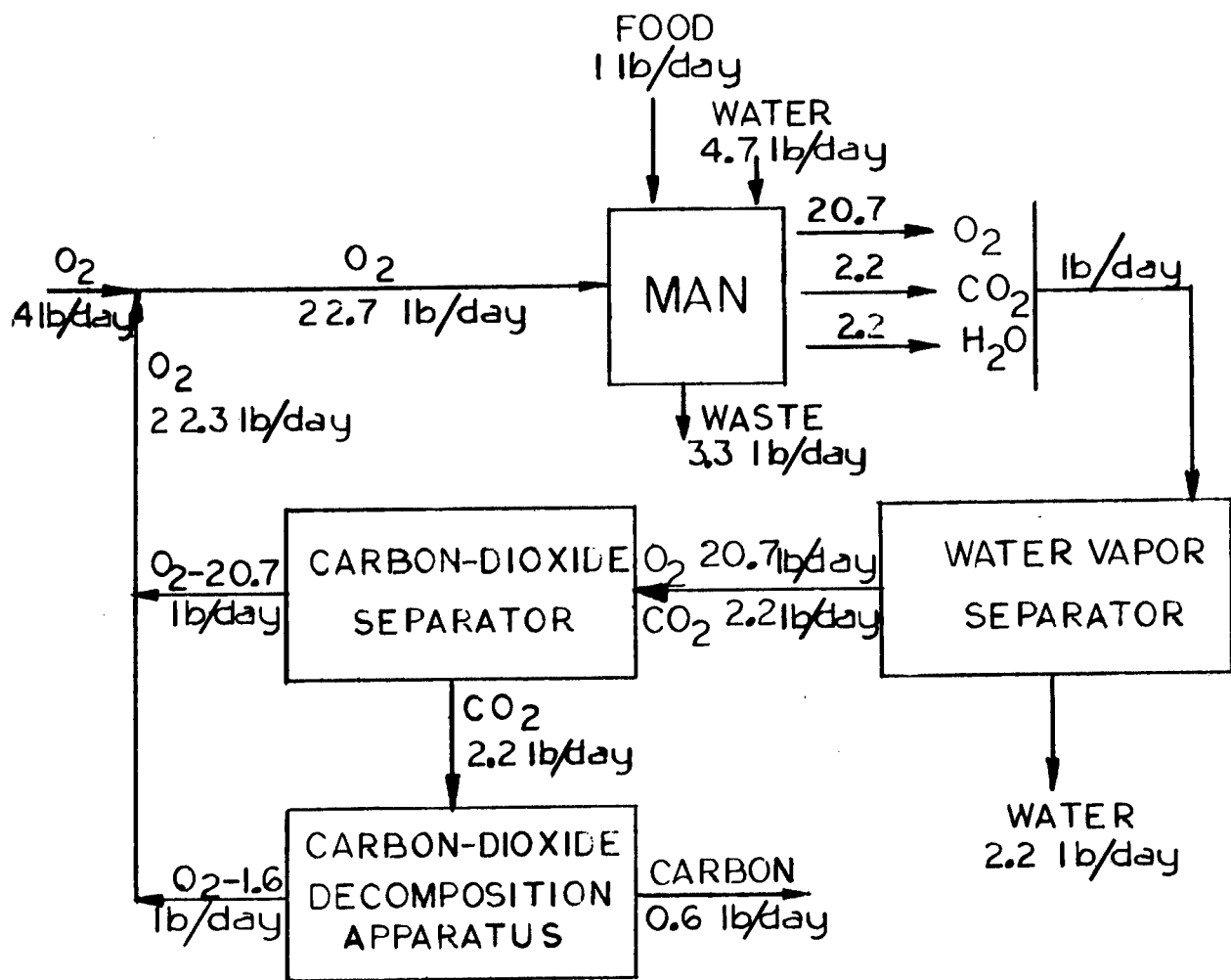


SYSTEM INPUT -

OXYGEN	2.0 lb/day
WATER	4.7
FOOD	1.0
TOTAL STORES	7.7 lb/day
CO ₂ SEPARATOR WEIGHT	
H ₂ O SEPARATOR WEIGHT	
ENERGY	

FIGURE 1.

IMPROVED CLOSED RESPIRATORY SYSTEM



SYSTEM INPUT —

OXYGEN	0.4 lb/day
WATER	4.7
FOOD	1.0

TOTAL STORES 6.1 lb/day
 CO_2 SEPARATOR WEIGHT
 CO_2 DECOMPOSITION
 APPARATUS WEIGHT
 H_2O SEPARATOR WEIGHT
 ENERGY

FIGURE 2.

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SAMPLING AND ANALYSIS OF ATMOSPHERIC CONTAMINANTS

By

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Programs are necessary to develop means of detection and quantitation of contaminants in closed ecological systems. This is of extreme importance when studies of man's physiological response to new parameters of atmospheric composition are being investigated. The study of various physiological parameters of long-duration space missions must not be influenced by contamination resulting from materials evolved by man, organic vapors outgassing from materials used in the construction of the system, and compounds originating from equipment used for the elimination of contaminants. Comprehensive contaminant studies may provide a key to necessary equipment in space vehicles to eliminate contaminants which may cause missions to be aborted.

Atmospheric contaminants have been identified, quantitated, and suspected from studies conducted on nuclear submarines, spacecraft and simulators (1, 2). At the USAF School of Aerospace Medicine, we have developed techniques for sampling and grossly identifying contaminants that tend to occur in simulators. This has included samples obtained not only during normal conditions, but also during abnormal conditions as characterized by a fire during one of the experiments (table I) (3).

Contaminant levels also have been explored in other ground-based simulators. For example, one experiment conducted at Boeing was terminated due to excess contaminant levels which produce physiological abnormalities. A complete knowledge of the contaminant problem is, therefore, important.

In order to determine effectively the toxicological aspects and establish threshold limit values (TLV) for the long-term closed ecological system, it becomes necessary to identify and quantitate the atmospheric contaminants within the system. This orients our discussion to techniques to obtain samples with sufficient concentration of contaminants to permit analysis.

SAMPLING

To determine the contaminants present, it is necessary to obtain adequate samples. The entrapment of a portion of the atmosphere to be sampled in a steel pressure cylinder is the simplest method of obtaining a

sample. A number of techniques may be employed. The sample cylinder may be evaluated and the sample taken by expansion of the atmosphere into the cylinder (fig. 1). The amount of sample that may be obtained is dependent upon the absolute pressure of the system being sampled. An alternate method requires the use of a pump (fig. 2) that will not add contaminants to the system from lubrication vapors. One which has been found to be satisfactory is a diaphragm pump, which utilizes stainless steel heads and fluorocarbon elastomer diaphragms. The maximum pressure obtained by this type of system is about four atmospheres. In the pressure sampling methods, there is no concentration of the contaminant contained in the atmosphere, resulting in a large diluent to a small contaminant ratio which increases the difficulty of analysis.

Another method of sampling the contaminants is the use of activated carbon. The atmospheric contaminants of the nuclear submarine and Mercury capsule have been sampled by this method. Activated carbon has an excellent sorptive capacity and is not easily desorbed of organic compounds by water at room temperature (4, 2).

Activated carbon, although it is an effective agent for the concentration of contaminants, has disadvantages. If the sorbent is not carefully protected after activation and sampling exposure, it may become extraneously contaminated and provide erroneous results. Catalytic reactions may occur which result in degradation of a contaminant, or in the interaction between contaminants sorbed on the carbon.

The use of a cryogenic fluid is effective in contaminant studies. A trap surrounded by liquid oxygen effectively traps contaminants from an atmospheric sample stream; however, the use of liquid oxygen is a hazard to the operators of such a system. The cryogenic fluid used could be liquid nitrogen, but the resulting entrapment would include oxygen. The trap would, as in the case of pressurization into steel cylinders, contain a large quantity of diluent to contaminant. The presence of oxygen would appreciably increase the probability of contaminant oxidation as the sample was raised to room temperature.

A trapping system consisting of two units in concurrent operation is effective in the concentration of contaminants at a temperature sufficiently high to eliminate the problem of liquid oxygen formation (fig. 3). A condensation-collection unit is established at a desired temperature by the regulation of the flow of cold, dry, nitrogen from a liquid nitrogen cooling unit. The system, however, is constructed of delicate materials and the sample must be stored at the temperature at which it is taken, or transferred to another container for storage or shipment (3).

Considering the advantages and disadvantages of the various methods for recovering contaminants from atmospheres, a multistage cryogenic trapping

system was developed at the USAF School of Aerospace Medicine which permits greater concentration of the contaminant than the pressurization approach.

The sample gas from which the contaminants are to be removed is passed through sample cylinders maintained at three different temperatures. An ice bath at 0°C ., a pulverized dry ice bath at -78°C ., and a liquid nitrogen bath regulated to -175°C . are used to fractionate the contaminants in the air stream. Materials not concentrated are oxygen, nitrogen, and compounds which have sufficient vapor pressure at -175°C . to pass through the system.

The air stream first enters a flow meter (fig. 4) and then passes to the first trapping cylinder which is maintained at a temperature of 0°C . with ice water. The gas, having passed through the ice bath trapping cylinder, flows through a heated inlet into the trapping cylinder maintained at -78°C . with pulverized dry ice. The pulverized dry ice requires occasional tamping to insure contact with the wall of the trapping cylinder. The gas then passes to a trapping cylinder maintained at -175°C . where many of the materials not previously removed from the gas stream are condensed. The remaining gas is conducted to the vacuum inlet of a circulating pump and exhausted by the pump into the atmosphere, or into a closed ecological system.

The trapping cylinders (fig. 5) are stainless steel with an internal volume of 150 cc. The cylinders are fitted with Swagelock connections, modified pipe fittings, and needle valves. Teflon and stainless steel are used throughout the system to minimize catalytic conversions and contamination of samples. A thermocouple is mounted through a tapped port in the bottom of the cylinder. Temperatures are monitored with copper-constantan thermocouples on a pyrometer calibrated in degrees centigrade.

In the -78°C . trapping cylinder, there is a rapid ice formation in the inlet tube. This formation, due to the temperature gradient along (or down) the entrance tube, is prevented by heating. The heater consists of 1/16 inch stainless steel rod inserted through a teflon-insulated fitting at the top of the cylinder. It is positioned in the center of the entrance tube and secured to the wall of the tube at the bottom. This connection serves as the electrical contact between the cylinder and the rod. Sufficient electrical energy is applied to the heater to prevent the formation of ice in the tube without affecting the operational temperature of the trap.

The final trapping cylinder is controlled at a temperature of -175°C . in order to prevent the formation and entrapment of liquid oxygen (-183°C . at standard pressure). The presence of liquid oxygen in the trap presents an explosive hazard for personnel handling the cylinders, and it makes available a supply of oxygen for degradation of the original contaminants and the formation of new compounds.

This cylinder is positioned with two glass-phenolic rings in a well which is surrounded by liquid nitrogen (fig. 6). A flow of dry, warm nitrogen from the bottom of the well controls the temperature of the trapping cylinder. The flow of gaseous nitrogen is regulated with a micrometer needle valve. One of the glass-phenolic rings covers the top of the well to maintain a positive pressure of gaseous nitrogen and prevent back diffusion of atmospheric air and liquid oxygen formation.

The well is positioned by a fitted lid for the Dewar flask. The lid also contains a vent, a well for the liquid nitrogen level sensor, and the liquid nitrogen filling device. The liquid nitrogen level control device was developed to simplify the operation of the system. The level controller uses a thermistor as a sensor so that a change in resistance of the thermistor, in or out of liquid nitrogen, results in a change in current. The change in current operates a control meter, which actuates a solenoid-operated liquid nitrogen transfer valve.

The multistage cryogenic trapping system will concentrate a compound if the vapor pressure at the trap temperature is less than its partial pressure in the sample stream. Partial separation of compounds occurs due to the different operational temperatures of the three traps. This separation simplifies identification and quantitation.

Table II depicts the distribution of several compounds as a function of the temperature at which they are expected to be concentrated in significant quantities. Substances are identified in each column according to the state in which they exist at that temperature, either as a liquid (L) or as a solid (S). Any material existing as a solid at a given temperature will not be found concentrated in a succeeding trapping cylinder.

Evaluation of the multistage cryogenic trapping system was made using a 255-liter chamber. The chamber was evacuated and 5 μ l. of acetone added. The chamber was pressurized to 760 mm. Hg with nitrogen. The amount of acetone in the chamber was determined by extraction of a sample into a 10 m. multipath, infrared cell, and quantitated by infrared absorbance.

The multistage cryogenic trapping system was connected to the chamber and operated for six hours. The flow through the system was indicated by a variable-area flow meter. At the end of the trapping period, the amount of acetone in the chamber and the trapping cylinders was determined by infrared absorbance. From the data, an indicated recovery of 90-102% was obtained (table III).

The portability of the system and the ease with which samples may be stored and/or shipped, facilitates contaminant studies in areas where, in the past, such tasks were not conducted because of lack of skilled personnel and equipment (fig. 7) (5).

METHODS OF ANALYSES

There are many analytical techniques that may be used in the analysis of contaminants. Procedures include the use of one or more of the following methods: infrared spectrum, mass spectrum, microwave spectrum, gas chromatography, pH, and nuclear magnetic resonance.

The techniques of infrared and gas chromatographic analyses were applied to the study of contaminants at the USAF School of Aerospace Medicine and at Melpar, Inc., where analytical assistance was provided. The most satisfactory system, due to the low concentrations that have been encountered, is flame ionization chromatography. The infrared instruments, using a 10 m., multipath cell have about the same detectability as the thermal conductivity cells of the gas chromatographs.

In addition to usefulness in trace contaminant studies, gas chromatographs have been amenable to the monitoring of major atmospheric constituents. A gas chromatograph was built in-house for the process stream determination of operational parameters in environmental simulators.

Under contract with the Air Force, Melpar, Inc., provided a "Six-Channel Readout for a Chromatographic Analysis of Space-Cabin Atmosphere" (fig. 8). The system displays the partial pressure of nitrogen, oxygen, carbon dioxide, water, and the total pressure in millimeters of mercury. Carbon monoxide is displayed as parts per million. The input analog voltages proportional to the partial pressures of nitrogen, oxygen, and carbon dioxide are obtained from the process chromatograph. The water vapor, carbon monoxide, and total pressure analog voltages are obtained from appropriate transducers. Information readout is by Nixie tube, and a digital printer, including date and time (6).

RECENT CONTAMINANT STUDIES

Air contamination studies have been carried out during animal experiments conducted in a space cabin simulator at the USAF School of Aerospace Medicine investigating oxygen toxicity. A simulator with an internal volume of 25.583 m.³ was occupied by subhuman primates, fowls, and rodents. The chamber was maintained at a temperature of 24° to 26° C. The total pressure in one case was allowed to follow ambient pressure for ground level baseline studies and also operated at essentially 100% oxygen at a total pressure of 258 mm. Hg. The animal cages were cleaned daily and the simulator constantly ventilated with ambient air at ground level and oxygen at 258 mm. Hg total pressure.

The atmosphere of the chamber was sampled on a periodic basis with the multistage cryogenic trapping system (fig. 9). The samples obtained at the three temperatures were shipped to J. Chaduet of Melpar, Inc. for analysis.

The analyses were accomplished with initial infrared examination and subsequent gas chromatographic study. In most cases, the concentration of contaminant was insufficient for detection by infrared spectra and identification was based on gas chromatographic retention time.

The contaminant and its highest concentration are reported at the conditions of the chamber in tables IV and V. The total amount of gas processed by the trapping system and the quantity of contaminant recovered in the traps were used to calculate the mg./m.³ in the simulator.

The chamber recently had been renovated and, based on prior studies of materials, certain of the contaminants could be attributed to the materials used in the process. During the selection of the materials to be used, the paint had been given intensive study. A detailed analysis of the paint, having an epoxy base, had been accomplished.

Painted panels had been placed in a 255-liter chamber and evacuated to 90 microns. The gas removed from the chamber was trapped by liquid nitrogen. The results of this trapping indicated that the solvents de-gas for a period of time after the paint has apparently cured. The solvents recovered were methyl isobutyl ketone, m-xylene, and toluene.

The freons are the result of the utilization of a freon cooling system rather than a freon-glycol exchanger in which the glycol would be used in the internal cooling coils. The source of the other contaminants has not been delineated.

PROPOSED EXPERIMENTAL INVESTIGATIONS OF ATMOSPHERIC CONTAMINANTS

In the past, in studies conducted in small volume, ground-based simulators, an inboard leak has presented problems in the study of man's contribution to air contamination. The delivery of a sophisticated multioccupant internal environmental simulator to the USAF School of Aerospace Medicine presents unique opportunity for the study of contaminants in a sealed environment without the inboard leak (fig. 10). The chamber was constructed with a double wall, the annulus being maintained at a pressure lower than the pressure of the internal area (fig. 11). The leak is thus outward from the occupied area as it is in a space vehicle. The internal chamber consists of two pressure vessels with a connecting sleeve contained in a single vacuum vessel. All of the pass locks, doors, plumbing, and electrical penetrations are designed to maintain the integrity of the system. The test cell has a volume of 27.75 m.³, the interlock 0.76 m.³, and the airlock 11.61 m.³.

A 28-day chamber operation will be performed. The operation will be divided into two segments of 14 days' duration. The first segment will establish background values of vapors from the materials in the cabin and from

previous occupancy by man. The second segment will provide information as to the type and amount of material contributed by man. A freezer-type fecal collection storage unit will be used. A portable urine container of sufficient size to accommodate a 12-hour accumulation by the 4-man crew will be used, and will be removed from the chamber when full. Carbon dioxide control will be accomplished by the conversion of LiOH to Li_2CO_3 . No toxin burner or carbon filter will be used.

During the course of the experiment, two cryogenic trapping systems will be in operation. Each system will be operated in two 10-hour shifts during each 24-hour period. The result is the production of four sets of samples per 24-hour period. Each set will consist of three samples, a gas fraction trapped at 0°C ., -78°C ., and -175°C . The samples will be distributed to several independent laboratories for analysis. In addition, an on-stream analysis system will be used to protect personnel within the chamber from exposure to compounds in excess of the maximum allowable concentration. This study is being accomplished in conjunction with National Aeronautical Space Administration, Manned Spacecraft Center, Houston, Texas.

SUMMARY AND CONCLUSIONS

Continued programs are necessary to develop means of detection and control of contaminants in sealed environments. This is of extreme importance when studies are being accomplished in simulators. The study of various physiological parameters of long-duration space missions must not be influenced by contamination resulting from toxic materials evolved either by man or his environment.

Further delineation of the contaminants contributed by man (and his materials) is required. Methods must be developed to eliminate excess levels of contaminants from the atmosphere of simulators and vehicles. Additional study is required to establish allowable limits at the pressures encountered in experimental and/or operational situations.

ACKNOWLEDGMENTS

The author wishes to express gratitude for the assistance contributed by Dr. B. E. Welch, Capt. W. Mabson and Airman Regula, members of the Environmental Systems Branch, and J. Chaduet of Melpar, Inc.

Table I. Compounds Isolated from a Sealed Atmosphere During a Fire

<u>Compounds</u>	
Acetaldehyde	Ethyl chloride
Acetone	Ethylene
Acetylene	Ethylene chloride
Ammonia	Formaldehyde
Benzene	Hydrogen chloride
Carbon dioxide	Methane
Carbon monoxide	Methanol
Ethanol	Methyl chloride
Ethyl ether	Nitrogen dioxide
	Propanol

Table II. Distribution of Compounds by Trapping Cylinder Temperature

Multistage Cryogenic Trapping Cylinder				Untrapped
0° C	-78° C	-175° C		
Water (L)*	Water (S)**	Acetone (S)		Methane
	Freon-12 (L)	Hydrogen sulfide (S)		Nitrogen
	Benzene (S)	Nitrous oxide (S)		Oxygen
Ethylene glycol (L)	Toluene (S)	Sulfur dioxide (S)		Hydrogen
	Ethylene glycol (S)	Trimethyl amine (S)		
	Diethyl ether (L)	Monoethyl amine (S)		
	Trimethyl amine (L)	Freon - 12 (S)		
	Monoethyl amine (L)	Freon - 13 (L)		
		Methyl mercaptan (S)		
		Carbon dioxide (S)		
		Ammonia (S)		
		Methanol (S)		
		Carbon tetrachloride (S)		

* (L) - Liquid

** (S) - Solid

Table III. Results of Preliminary Evaluation: Efficiency and Recovery

Amount of Acetone in Chamber (mg)		Amount of Acetone in -175° C Trapping Cylinder (mg)		Percent Recovery	Percent Efficiency
Initial A	Final B	Measured C	Predicted D	$\frac{C}{A-B}$	$\frac{C}{D}$
4.072	2.610	1.50	1.702	102.6	88.1
4.210	3.117	1.07	1.457	97.9	73.4
4.210	2.900	1.19	1.457	90.8	81.7

Table IV. Animal Experiment Contaminant and Concentration at Ground Level

	Highest Concentration Obtained ₃ (mg/m ³)	Maximum Acceptable Concentration (mg/m ³)
Carbon dioxide	421	9000
Freon-12	4.93	4950
Freon-114	4.45	7000
Freon-22	3.06	-
n-Pentane	0.39	2950
Diethyl ether	1.67	1200
1,1-Dichloro ethylene	0.69	-
Acetone	2.28	2400
Methyl acetate	0.03	610
n-Heptane	0.21	2000
Methyl ethyl ketone	5.77	590
1,1,1-Trichloroethane	0.14	1900
Methanol	0.35	260
Benzene	0.01	80
Ethanol	1.70	1900
Ethylene dichloride	0.11	200
Trichloro ethylene	0.02	520
Chloroform	0.72	240
Methylisobutyl ketone	0.18	410
Toluene	0.42	750
Tetrachloro ethylene	0.10	670
M-Xylene	0.63	870

Table V. Animal Experiment Contaminant and Concentration at Reduced Pressure

Contaminant	Highest Concentration Obtained ₃ (mg/m ³)	Maximum Acceptable Concentration ₃ (mg/m ³)
Acetone	0.02	2400
sec-Butyl alcohol	0.05	300
Carbon dioxide	167	9000
Chloroform	0.005	240
Cyclohexane	0.003	1400
Ethanol	0.005	1900
Freon-22	0.03	-
n-Pentane	0.05	2950
iso-Propyl alcohol	0.10	980
Methanol	0.02	260
Methyl isobutyl ketone	0.04	410
Methylene chloride	0.03	1750
Tetrachloroethylene	0.006	670
Trichloroethylene	0.003	520
Toluene	0.04	750
m-xylene	0.23	870

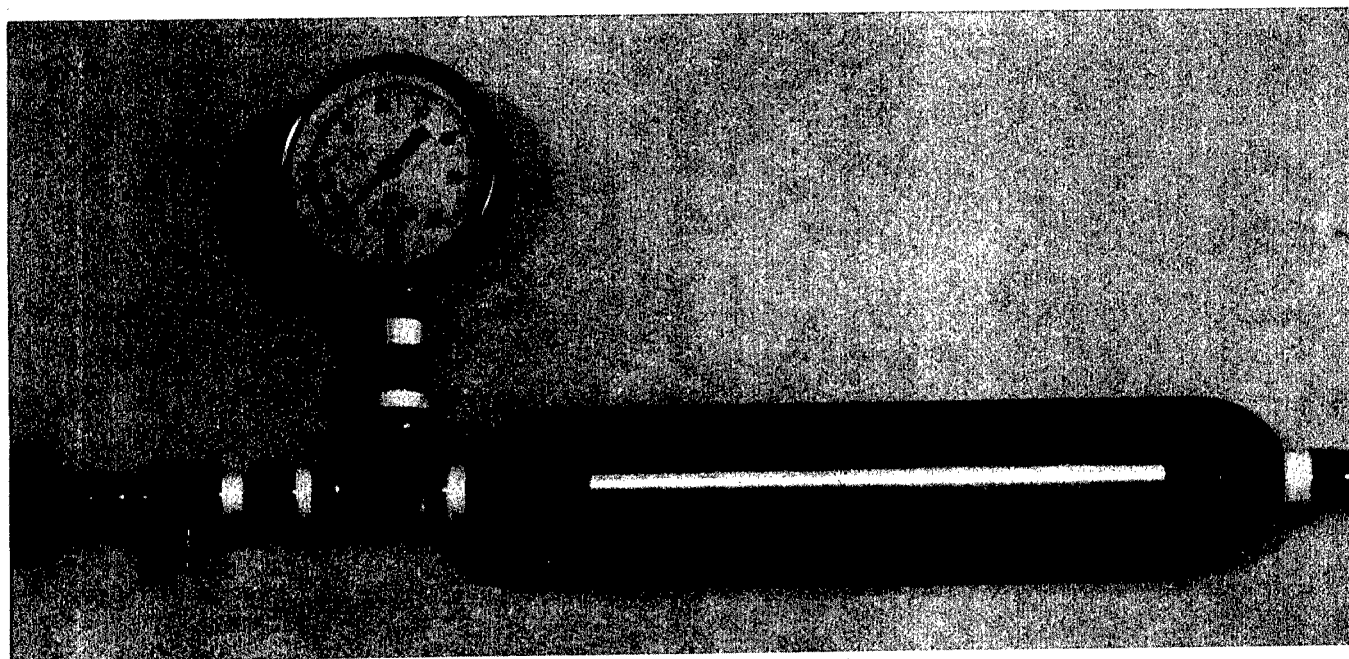


FIGURE 1. SAMPLING BY EVACUATED CYLINDER

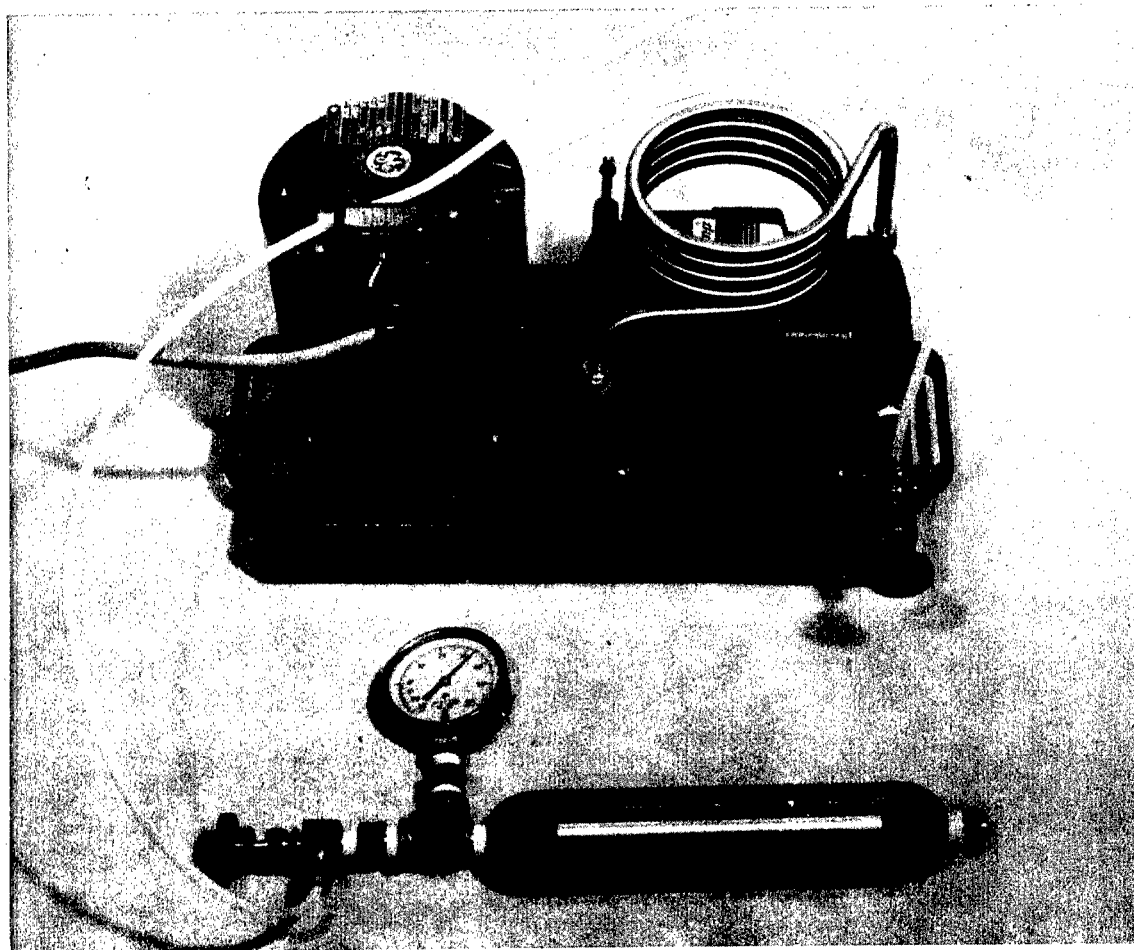


FIGURE 2. SAMPLING BY PRESSURE PUMP AND CYLINDER

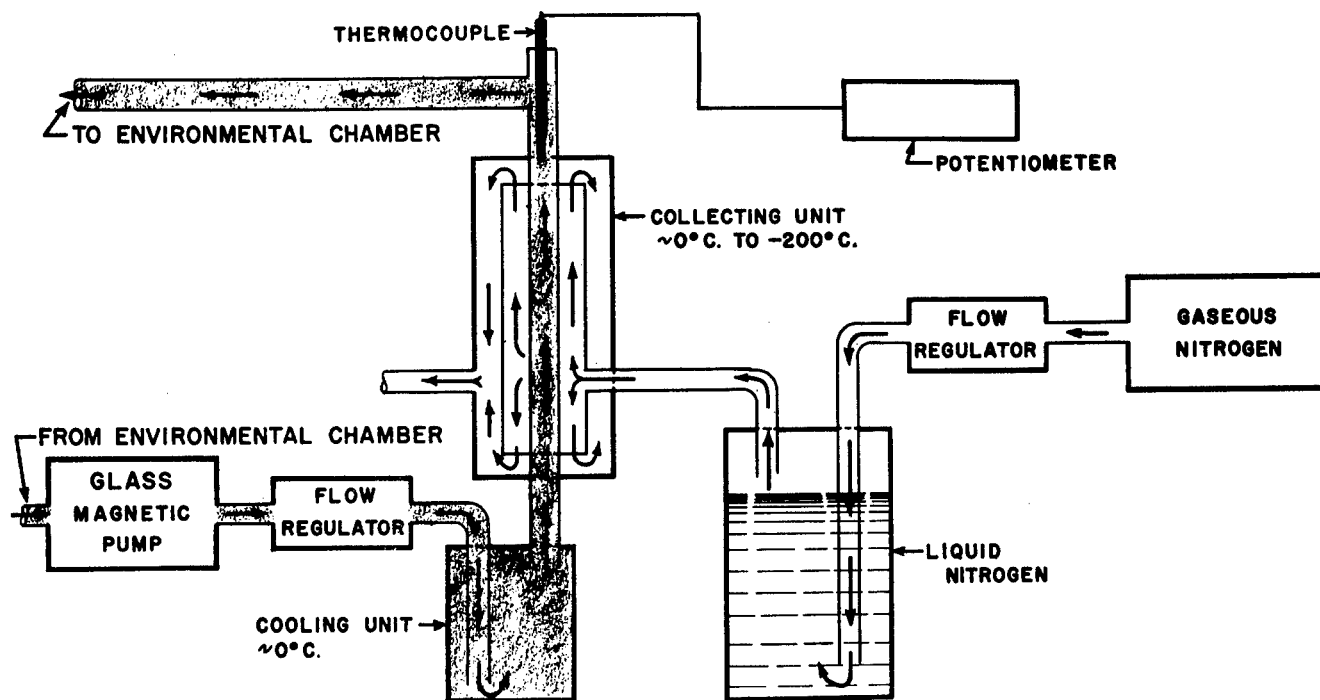


FIGURE 3. ISOLATION SYSTEM FOR MICRO-CONSTITUENTS IN A SEALED ATMOSPHERE

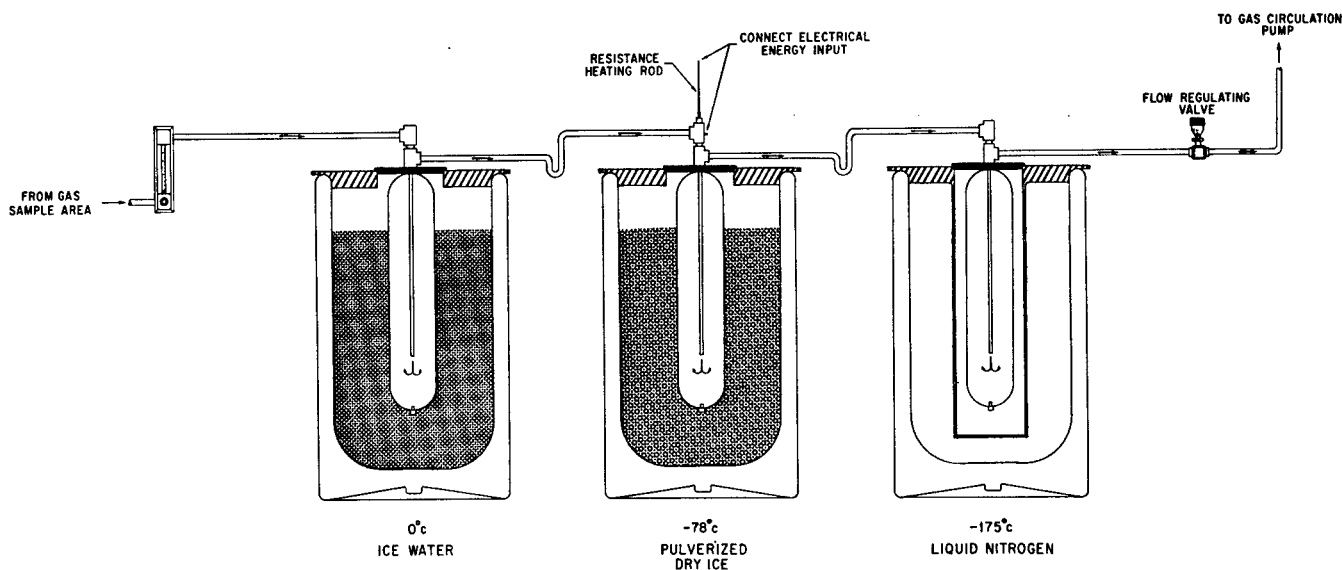


FIGURE 4. GAS FLOW PATH FOR MULTI-STAGE CRYOGENIC TRAPPING SYSTEM

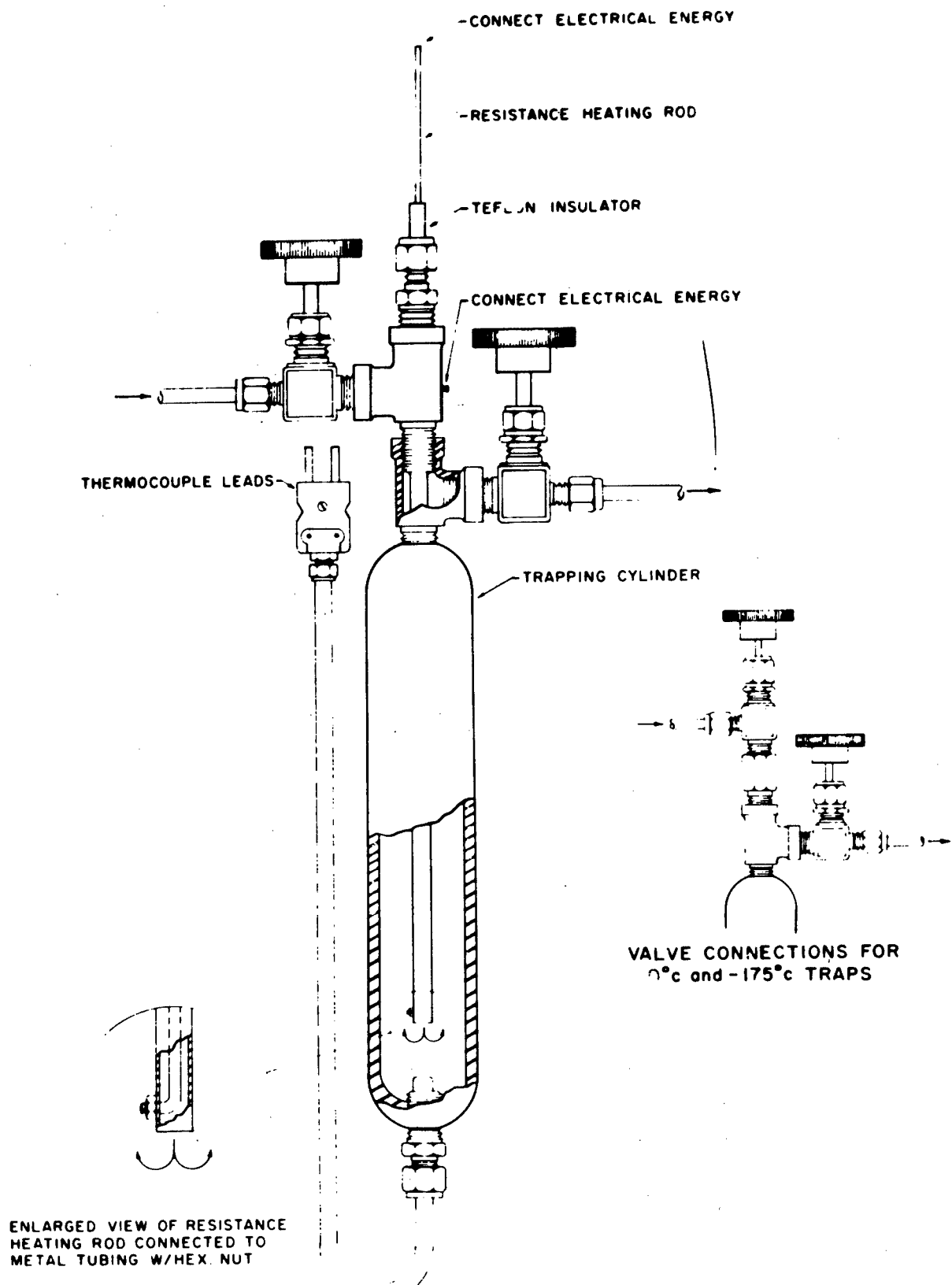


FIGURE 5. MULTI-STAGE CRYOGENIC TRAPPING SYSTEM

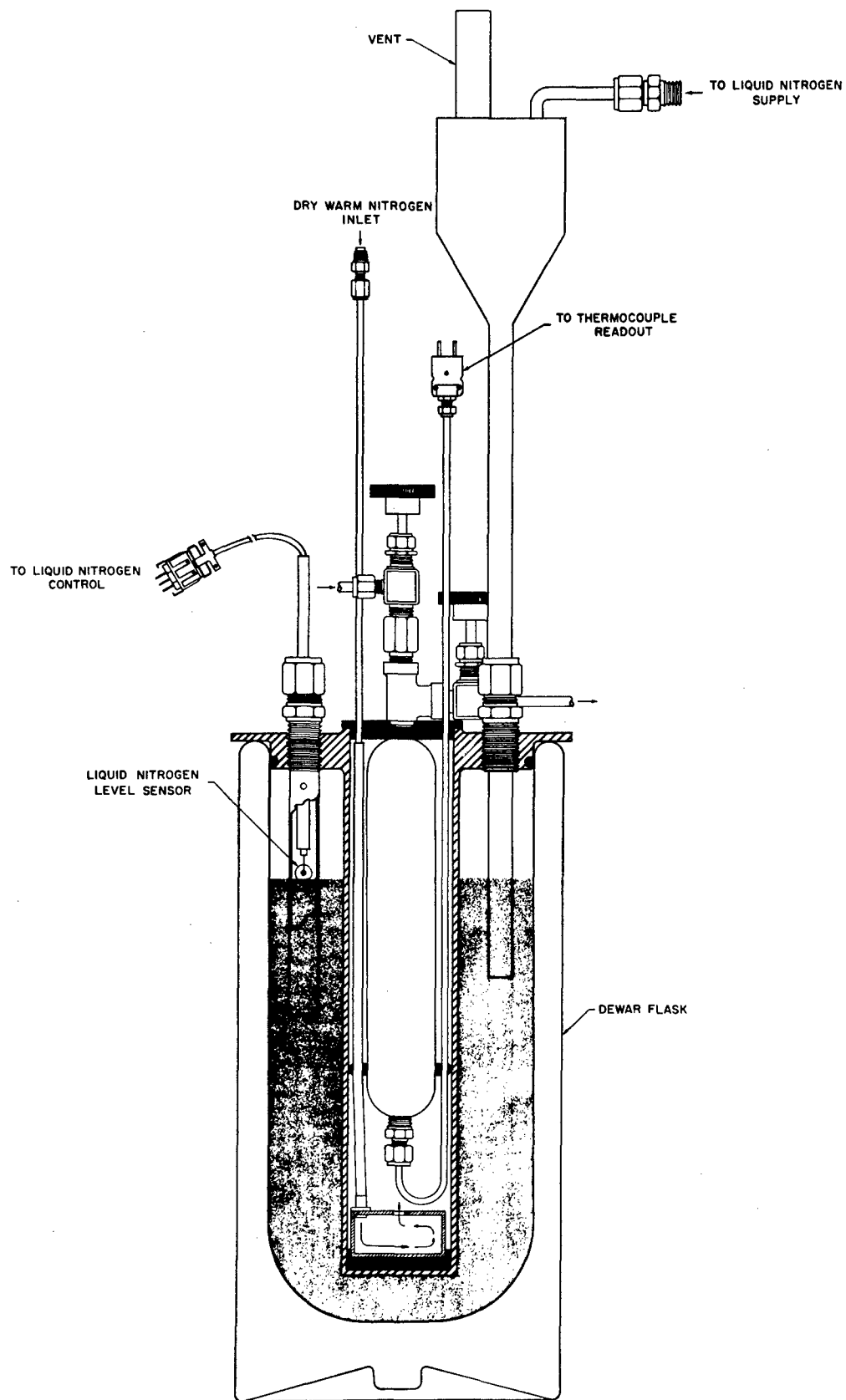


FIGURE 6. LIQUID NITROGEN TRAP (Cross Section)

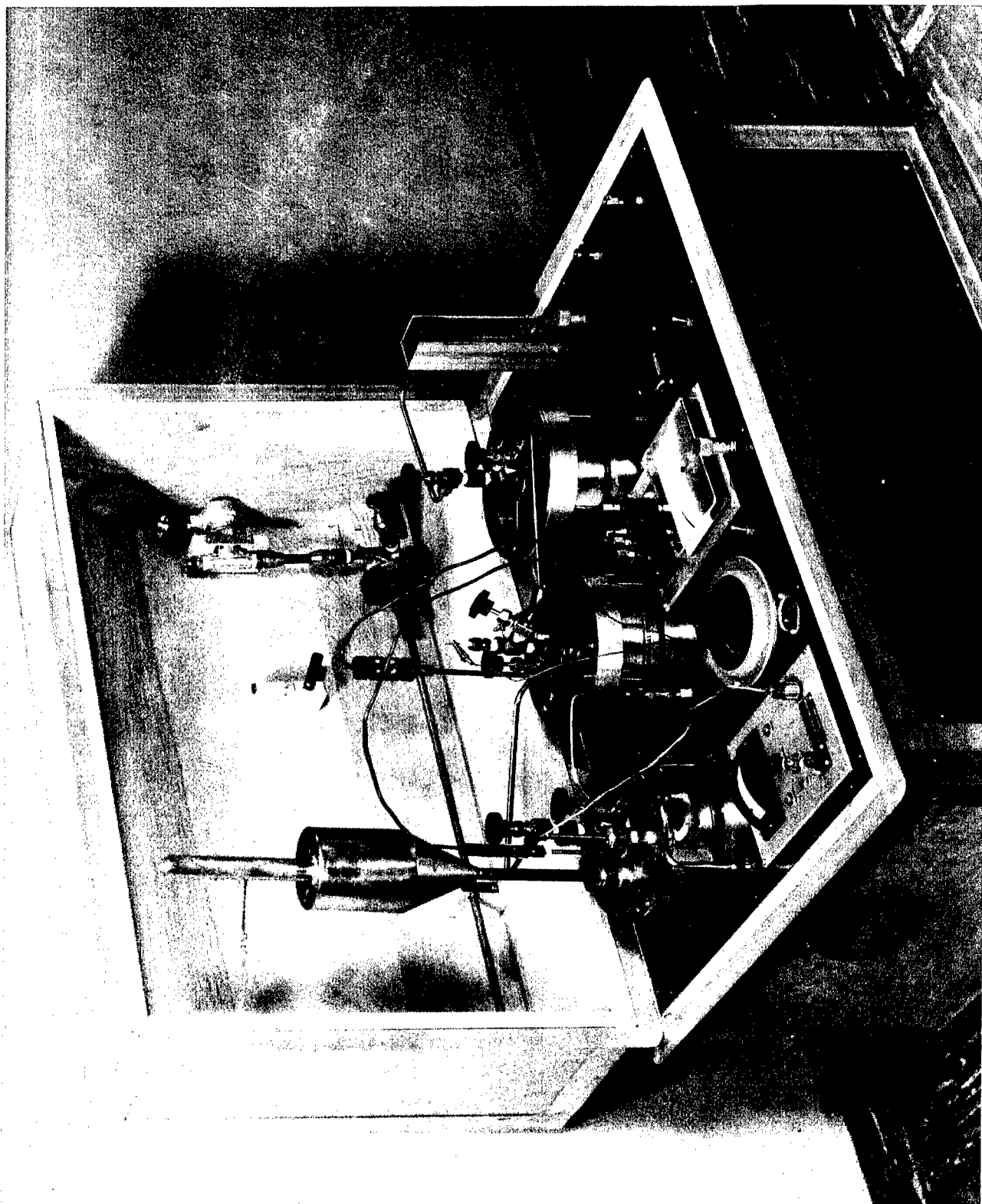


FIGURE 7. PORTABLE MULTI-STAGE CRYOGENIC TRAPPING SYSTEM

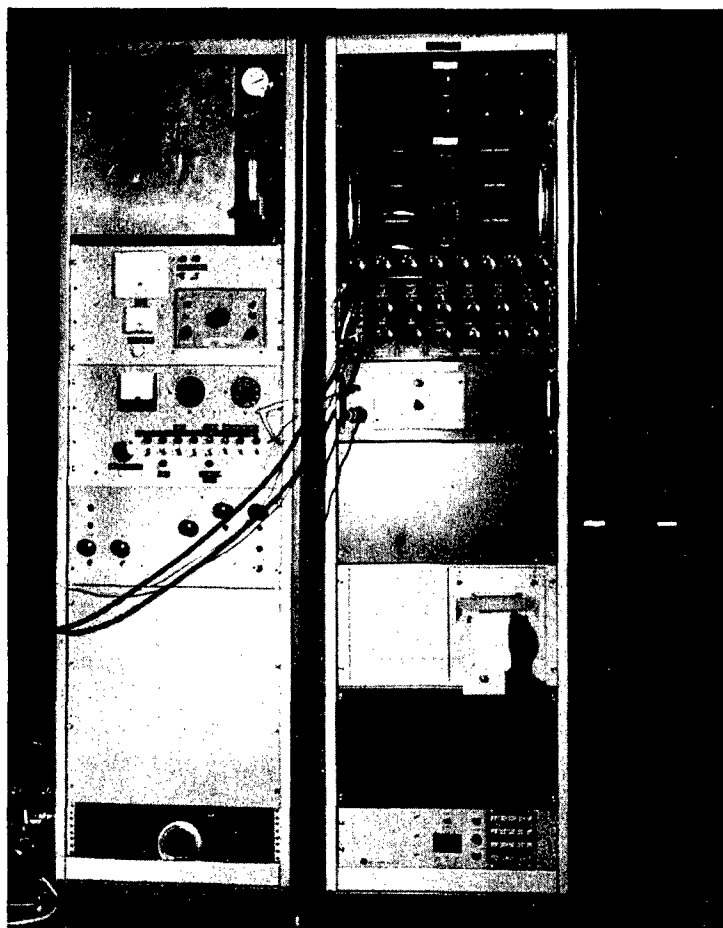


FIGURE 8. SIX-CHANNEL READOUT FOR A CHROMATOGRAPHIC ANALYSIS OF SPACE CABIN ATMOSPHERE AND PROCESS STREAM GAS CHROMATOGRAPH

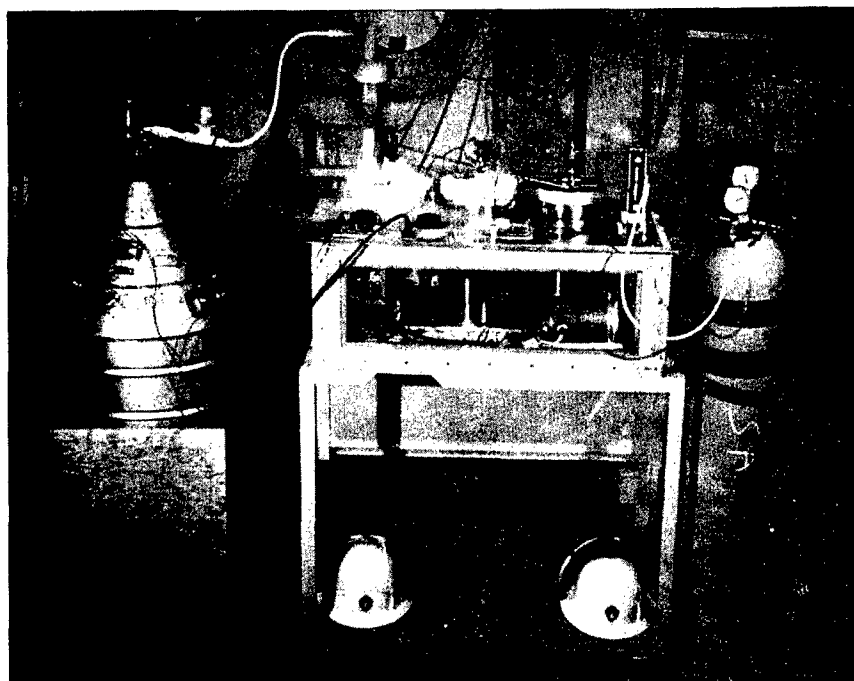


FIGURE 9. MULTISTAGE CRYOGENIC TRAPPING SYSTEM SAMPLING CHAMBER ATMOSPHERE

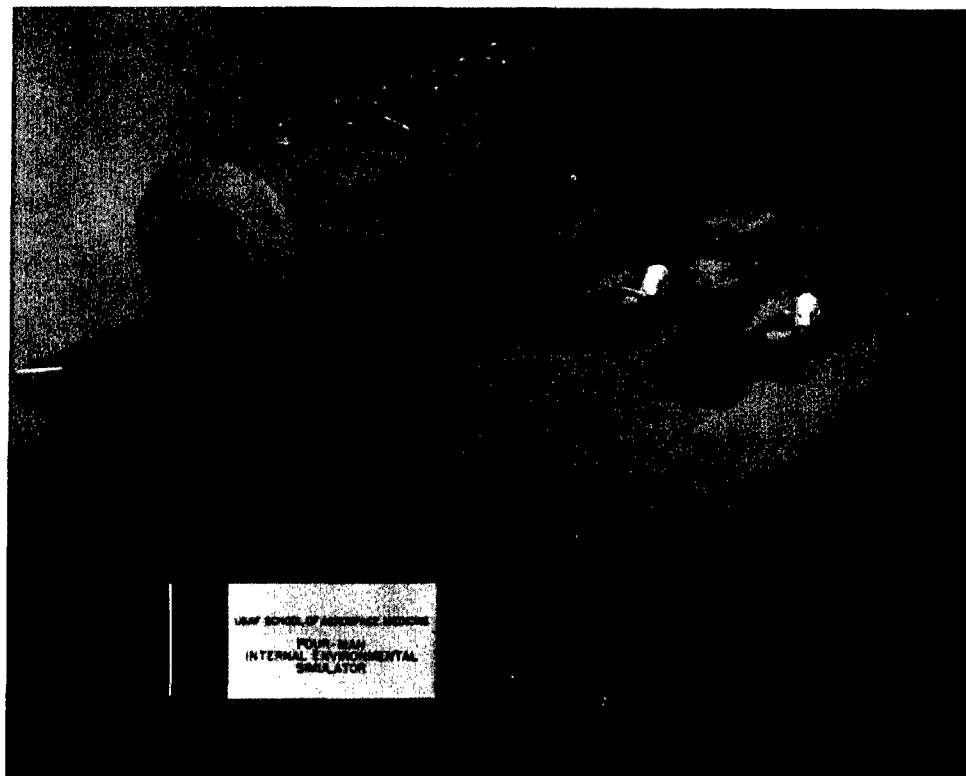


FIGURE 10. SOPHISTICATED MULTIOCCUPANT INTERNAL ENVIRONMENTAL SIMULATOR

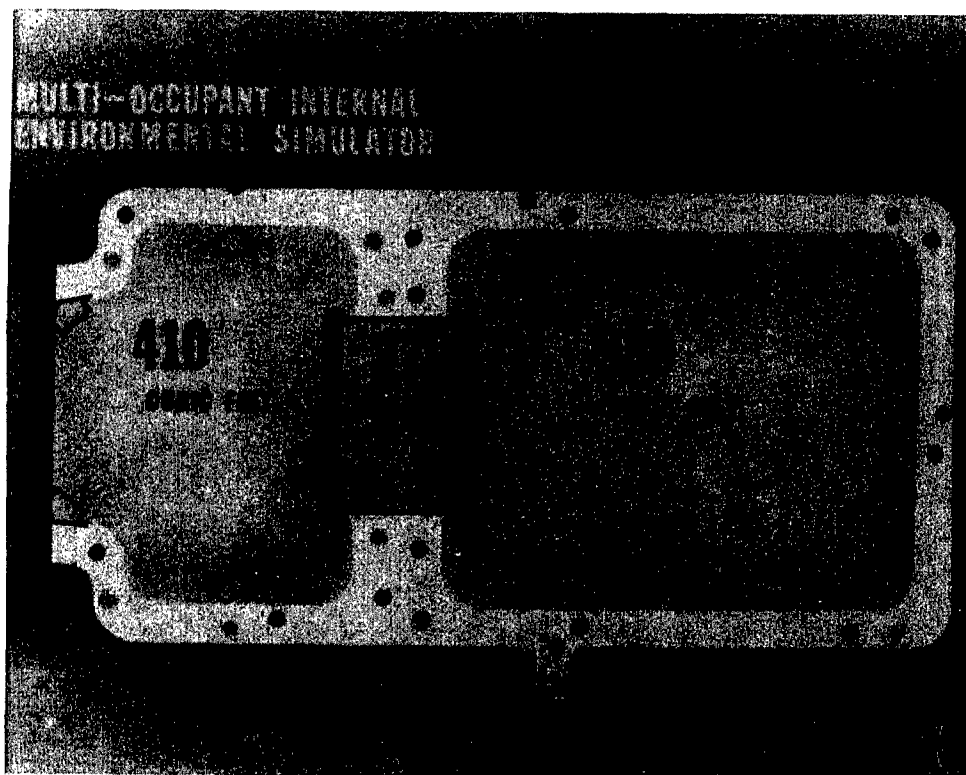


FIGURE 11. DIAGRAMMATIC REPRESENTATION OF MULTIOCCUPANT INTERNAL ENVIRONMENTAL SIMULATOR

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The frontispiece illustrations were prepared by the USAF School of Aerospace Medicine and originally shown as part of this presentation.

QUESTION AND ANSWER SESSION

Q. Dr. Roth, Lovelace Foundation: I'd like to ask Mr. Conkle what the abort criterion is in the run being scheduled.

A. Mr. Conkle: This is something still to be finally established. It is under consideration at the present time. There will be more meetings before the experiment is actually in progress.

Q. Mr. Wilson, Boeing: I'd like to ask Mr. Roundy if they have done any work on successive degassing of cabin materials.

A. Mr. Roundy: No, sir. My particular group hasn't, although there may be people here in the laboratory who have done such work. Have you done any of this, Anton?

DR. THOMAS: No

MR. WILLARD: (Honeywell) We have done some limited work in this area, particularly with the finishes where the contaminants that we first encountered consisted largely of carbon monoxide and some solvents. Some finishes successive to degassing operations did not significantly change the ultimate gassing characteristics of the materials, particularly in the area of CO production. In other cases, it did considerably improve the gassing characteristics. One notable exception was a particular silicone enamel, which, under our attempts at degassing, continued to give off copious quantities of benzene time after time after time, following successive degassing studies. There is conflict with this particular material, however, so we have not been able to confirm this with other investigators.

DR. HARRIS: I think Mr. Pustinger might have some comments to make on that, do you John?

MR. PUSTINGER: I will be the next speaker, so I'll wait.

CAPT. CARTER: (Aerospace Medical Division) Dr. Harris, in your proposed test for qualifying materials, you mentioned that you were going to have a simulation technique for generating contaminants to be studied for those which passed -- I think it was for those which passed the first three stages in your test. Before you can simulate generation of the contaminant, you have to have some indication of the quantities of the materials you have in your spacecraft, as well as your surface areas that you will have in your

spacecraft. Consequently, this means that you will just about have your spacecraft designed. Will you not lock yourself in?

DR. HARRIS: No, I don't believe so, because what we want to do is start an outgassing study rather early, and then try to lock-in with the designers and try to keep pace with them.

To perform a toxicological study on a group of materials about which you have no information in terms of weight and surface area may be meaningless. But we are hoping to be able to obtain enough prior information so that at one point in the course of the design we can say, "There is something that we have information on about generation and decontamination rates. Can you, as design engineers, provide us with surface areas, and with weights to determine whether this is an acceptable material based upon these criteria?"

CAPT. CARTER: I appreciate your point there. One other general comment -- I think that due to the differences in the material that you would get from a supplier, you must go through your atmosphere validation program once you have the spacecraft designed, no matter what.

DR. HARRIS: That's absolutely true, as you observed in the last figure.

COL. WHITE: I'd like also to ask your position and your formula for toxicology control of specialized instrumentation aboard spacecraft, to handle any problem that you can assume you haven't solved by the first five or six steps that you propose.

DR. HARRIS: I cannot answer that directly. What we are attempting to do, as I explained, is to get ahead of the design engineers, and to try to control materials outgassing and to adapt the contaminant control system to provide for the removal of the major portion of the contaminants. Now this, I think, is the question you're asking, is it not?

COL. WHITE: I think that what I was getting at is that you have proposed a logical step by step program to keep them from getting in. This is on the assumption that you are 100% successful. You've also tried to predict what your problems will be, in fact, by your dynamic simulation, which will allow you to prognosticate as to how it will go in the flight. It would seem to me to be an equally important area after the fact now, and during the flight, to demonstrate that you are on track and you are following the prescribed schedule, if you wish, as a flight safety item.

DR. HARRIS: In Apollo, for example, we are anticipating an on-board gas chromatograph. This will follow the development of a number of possible contaminants during flight.

MR. MASON: (NASA) I believe you mentioned that you had a program in conjunction with Wright-Patterson on cabin materials. Would you care to comment as to what specific materials you are investigating there?

DR. HARRIS: We estimate we will determine the outgassing products of approximately 50 spacecraft materials per year, and the outgassing rates over 30-, 60-and 90-day periods. We are going to examine short-term (7 days) toxicity on an estimated 400 materials. These will be performed in groups of 10 after the first group is completed. The first group will be in excess of 60 materials. They are going to be run on a unit weight basis, and will be outgassed using only two species of animals. We are using a relatively crude test system in an attempt to get abreast of the design. The materials to be studied will be selected on the following basis: those materials which are used in the spacecraft, but have not been tested, will get first priority; those which are being used in the spacecraft and are in the process of being tested will get second priority; and those which have been tested and are being used in the spacecraft will have last priority.

Now, to enumerate all of these materials, I'm afraid I can't.

DR. SCHWARTZ: (Grumman Aircraft) I'd just like to make a comment. I'd like to say it isn't as hopeless as it may seem about this business of getting locked-in. Although our spacecraft actually isn't built, we're making the Lunar Excursion Model (LEM). In addition to the testing of materials prior to use in the spacecraft, which you've seen in the chart, we are going to supply NASA a monthly list of quantities of non-metallic materials as they are assembled in the spacecraft; and, during the construction of the spacecraft, each month there will be a monthly list of quantities and the surface area of these materials which will go to Dr. Harris, which we hope will prevent us from getting into the problems that others may have gotten into in the early work by locking the vehicle and not carefully analyzing the thing; so I feel that, with this monthly list of the quantities, we should be fairly safe.

DR. STOKINGER: I'm just a little worried here. We have a lot of engineers and we see displayed on the board a great many materials that have potential toxicity, and I'd like to provide just a little possible perspective to these engineers in this way. I wonder how many of you have seen the chromatographic analysis of the aroma from a hot cup of coffee. You'll find that many of the materials, and I think they identified 30 from this aroma, are in many instances identical with some of these products you have listed here. Among them are methyl alcohol, many ketones, and even carbon disulfide, and, of all things, diacetyl, a highly irritant material, things you wouldn't feed to your grandmother, of course, but you do. The thing is this -- that the body, of course, has ways and means of metabolizing these things, provided they are in small enough concentration, and that's the real problem here. I'd like to impress this upon the people so they don't get unduly worried and concerned about this.

DR. HARRIS: This, I think, is a rather important point. You do have a rather good decontamination system in man himself; but I think we're concerned primarily here with the levels which will achieve toxicity. The situation in the space atmosphere is not directly analogous to a cup of coffee, but it is a very well taken point.

MR. WILSON: (Boeing) I would like to ask what sort of a relationship, with regard to the choice of materials and their evaluation, will exist between, let's see, your group and the contractor who is going to build a capsule. In other words, what responsibilities would we have as a contractor in choosing this material? Would you feed us information to allow us to choose the materials, or how would it work?

DR. HARRIS: Wherever possible, we will try to feed you information to choose materials. There will have to be a very close working relationship between the toxicity group and the design engineer, there's no question about that. What we are hoping to do is to avoid a statement which says the spacecraft manufacturer shall guarantee a non-noxious atmosphere. This becomes difficult to handle.

Q. Dr. Thomas: Will there be any feedback from the manufacturers and from the engineers who build the little black boxes on last minute changes and substitution of material?

A. Dr. Harris: This has not been established, but it should be in the statement of work for the next generation spacecraft. There should be an effective means for informing the spacecraft manufacturer and NASA, whichever is responsible, of last minute changes.

MR. WILLARD: (Honeywell) I might comment on the approach that we have been following at Honeywell for this particular problem. About last May, we embarked on a top-down review of all the Apollo stabilization and control system drawings. I think there were some 10,000 drawings involved. We tabulated on punch cards all exposed organic materials in location, material designation, weight and area. We put ourselves into the engineering change order loop and we have a draftsman assigned to the task of maintaining the punch card files. We print out and update at periodic intervals and transmit this to the prime contractor. We can sort this by device number, by material category and tabulate total weight for each material. This is maintained, as I say, on a current day to day basis.

DR. SCHWARTZ: (Grumman Aircraft) We've probably solved the black box problem. We've sealed almost every black box individual sub-component -- hermetically sealed it with the sole exception of the environmental control system. Of course, if we've sealed the black box from the cabin, you don't have to worry about getting any noxious gases to the cabin area.

DR. HARRIS: This is essentially the point I brought out. I think I said that there are some of these items which will have to be sealed off from the spacecraft habitable area.

COL. WHITE: (USAF) I'd like to comment on that. This is true, this is a day of sealing black boxes; but there is also a second wave coming, and that is that man is going to be injected into the repair and maintenance, or he will be injected into these black boxes, and, either way, make the decision of having to replace them in toto or going into them and replacing them in part, and I think we shouldn't be smug and complacent at this point.

DR. HARRIS: That's right.

DR. DU BOIS: I'd like to ask about a different class of compounds, liquid and solid aerosols, whether the exposures that are contemplated have a system for measuring particulate matter or liquid aerosols. Secondly, whether the spacemen when they see the sunshine come in the window will see the dust beam in the sunshine or is the atmosphere clear?

DR. HARRIS: The answer to both questions is number one, there is no method used for determining dust particles. One of the Apollo experiments is to carry a nephelometer. Secondly, the atmosphere is not totally clear, there are obviously dust particles. I think this has been evident in the past.

MR. GISCLARD: (W-PAFB) I'm here more or less as an observer. I have brought this question up before to others. We talked about it last night, and I'm going to throw it out at this time. That is, with the sophisticated instrumentation that we are using in all this investigative work, has anybody given any thought to the possibility of going back and using classical chemistry from this standpoint? It just seems to me that some kind of support equipment could be used in the event that some of these sophisticated instrumentations did not work. I'll just take one example. I will trust a 50¢ carbon monoxide tube that I have in my possession, that is 20 years old, to tell me what the amount of carbon monoxide is before I would trust a \$5,000 instrument. It has been tried, proved and tested, and it is good. It was developed for the Air Force during the war. Now, my point is, are we eliminating classical methods of chemistry, are we eliminating them entirely, so that we have to go completely to sophisticated instrumentation for all of our answers, or is there some realm where we can bring classical, chemical methods into some kind of supportive equipment, some kind of kit, something to hang underneath the seat, perhaps, and if you had to use it, use it. I don't know, I'm just throwing the question out.

DR. HARRIS: My answer to that sir, is yes sir.

MR. GISCLARD: Good.

DR. HARRIS: Sensitive tapes are being considered in order to observe the development of particular atmospheric contaminants within the craft. This is partially an answer to your question, Col. White.

Most of the ground testing is done with gas chromatography, mass spectroscopy and other modern sensitive instruments. We can compare the results obtained by highly sophisticated instrumentation with simple gas samplers and sensitive tapes.

MR. HOLDEN: (Grumman Aircraft Engineering) In support of the gentleman regarding the use of classical methods, of course we use complex instrumentation in our outgassing studies; but, in addition, we are using such classical methods as Nesslerization for the determination of ammonia; we detect hydrogen cyanide as an outgassing product by using a classical method employed in the sewage industry; we are using acid-base titration procedures for various amines and carboxylic acids -- so we have not forgotten the classical methods at all.

CAPT. CARTER: (Aerospace Medical Division) I think one of the things that comes to light here is that in most cases of such materials as carbon monoxide and carbon dioxide, for which you do have comparatively reliable classical chemical methods, you want continuous readout. Consequently, you're going to have a pretty good quantity of reagent materials to carry aboard the spacecraft. In addition, in some of the complex instrumentation, as you call it, you can get readouts of a lot more than one chemical.

DR. HARRIS: All levels of chemistry are used in spacecraft atmospheric evaluation. We can't depend entirely upon gas chromatography, mass spectroscopy, and so on. You can't let any phase of analytical chemistry go by the board and say "forget it, we've got something better now".

DR. SCHOLZ: (IIT Research Institute) Has any consideration been given to the pinpointing of a malfunction based upon the characteristics of gas odors evolved?

DR. HARRIS: I can't answer that question.

DR. SCHOLZ: Do you think such a technique would be valuable?

DR. HARRIS: Perhaps it would be.

MR. CHOLAK: (University of Cincinnati) I've heard about the gassing off of all the organic materials, but I haven't heard anything about man himself. What about that?

DR. HARRIS: This was the basis for Mr. Conkle's talk. Many spacecraft materials will gas off very rapidly, drop down to practically nothing,

and then go on continuously from there at very low levels. But there is one continuous source of off-gassing products in the spacecraft; that is man. He is something that we cannot remove, so we're trying to determine his contribution to the total outgassing.

MR. CHOLAK: It hasn't been done yet? You don't know the organic materials there are in man?

DR. HARRIS: There are lists of such materials. There is one list of roughly 400 materials that are supposedly given off by man. I think we want to examine this directly, using man sealed in an appropriate atmosphere. With Mr. Conkle's sophisticated system, we should obtain some interesting and enlightening results.

MR. LANDIS: (Hughes Aircraft) I might add that in answer to this question, there is at the present time being compiled a rather detailed chromatographic catalog of all of the materials that are intended to be used on the Apollo Command Module; because of the complexity of some of the components that are coming off as gas-off products, it's almost impossible to identify all of the products in any reasonable length of time, or with any reasonable amount of money. So this catalog of chromatographs will be available; and, once the Apollo Command Module is actually built and tested, they will then compare the actual chromatographs they get from an actual testing of the atmosphere in the Command Module, and then if some particular material appears to be giving trouble, a particular contaminant appears to be responsible for toxic effects, they hope to use this as a means for pinpointing what is the guilty culprit. This is a very complex problem because of the fact that we have a sampling problem. A typical example -- you can get a sheet of material, let's say an epoxy laminant (and this is going to be used in the Apollo Command Module), you take a sample out from one portion of the laminant, and you get a chromatogram that indicates it's full of garbage, and then you take another sample out from a different part of the laminant, and it turns out to be pretty clean. The problem is just fantastic in just being able to pinpoint a particular material as being the guilty culprit, and this is what we're up against.

DR. HARRIS: We have time for just one more question.

MR. SAUNDERS: (Naval Research Lab) I'd like to go back to a couple of questions and comments on pinpointing sources of trouble of contaminants. On one case, in a Mercury atmosphere, there was some hexamethyltrisiloxane recovered. In that particular flight, there was reported a failure of a bearing, which had been lubricated with a silicone grease. This is the only flight in which the silicone compound was ever recovered, and that might indicate that malfunction had been pinpointed. In the case of submarine atmospheres, there have been occasions when material observed in the atmosphere has indicated various malfunctions in some of the contaminant

control equipment, or not necessarily a malfunction, but a change, in the operating conditions of the equipment. For instance, Hopcalite burners. The temperature at which these things are operated has a definite effect on the type of material sometimes found in the atmosphere. About the materials which are found in general in chamber atmospheres and space cabin atmospheres, it's true that man evolves a considerable number of contaminants; but it must be that these occur in the atmosphere at very low concentrations. I've never found it possible to assign any of the things which I have detected in these atmospheres to man himself. Most of them seem to evolve from equipment.

ANALYTICAL TECHNIQUES FOR IDENTIFICATION OF GAS-OFF PRODUCTS FROM CABIN MATERIALS

By

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INTRODUCTION

Contamination of man's breathing atmosphere has been a problem throughout his existence. Years of extensive research on air pollution have been performed in attempting to determine the nature of the contaminants and their toxicity. Only recently, when man has been confined for lengthy periods in submarines and space vehicles, has the need been recognized to establish potential sources of contamination from the materials of construction of that environment.

The identification of trace contaminants and the establishment of toxic levels is extremely important. Buildup of contaminants through oxidation and hydrolysis reactions, and gas-off of solvent or adsorbed materials, not only creates hazards from individual components, but provides a multi-component system with potential synergistic effects.

Considerable analytical data on trace contaminants have been obtained from the atomic submarine programs and the various space cabin simulators. However, very little information is available on the specific sources of contamination, that is, the individual materials that make up these vehicles or chambers.

Some evaluation programs have based acceptance or rejection of candidate materials on olfactory sensing, i.e., sniffing for odors. Others have induced thermal decomposition, measured weight loss, and to a limited degree characterized the gas-off products by spectroscopic means. Unfortunately, little information useful to the toxicologist has been obtained.

Under the sponsorship of the Toxic Hazards Branch at Wright-Patterson Air Force Base, a program was initiated at the Dayton Laboratories of the Monsanto Research Corporation to identify the gas-off products from a variety of candidate space craft materials and to estimate the concentration and gas-off rates of these potential space contaminants. Eventually 50 materials will be tested and approximately 1000 analyses will be performed. Only a portion have been completed at this time. A listing of general types is presented in Table I.

All materials are commercial products provided by the Government. Some are partially-fabricated sections from the Gemini program, whereas others require preliminary preparation.

The experiments are designed to simulate normal conditions, 23-25°C and 20-40% relative humidity in two atmospheres, air at normal atmospheric pressure, and oxygen at 5 psi.

To obtain a measure of gas-off rate, all candidate materials are stored in 9-liter, borosilicate glass chambers for periods of 30, 60 and 90 days. Sample charges range from 10 grams to 100 grams. Duplicate chambers are prepared for each test. A typical chamber and helix for isolation and concentration of gas-off products are shown in figure 1. Whenever possible the candidate materials are placed into the chambers in the same state as received. The paints, inks and coatings are applied to aluminum foil substrate and cured according to the manufacturers' directions.

Since the freshness of the sample could easily influence the type and amount of gas-off product, the 30-day test chambers are analyzed, purged of their environments and recharged with air or oxygen. After an additional 30-day period, the atmosphere of the chamber is again analyzed, purged and recharged. Thus, the total accumulative storage time is 90 days.

Due to the wide range of sample type and levels of gas-off components, several different sampling techniques and analytical methods are used. Four basic analytical operations are performed:

1. Analysis for carbon monoxide and methane
2. Direct gas chromatography analysis of gas-off products
3. Mass spectrometry analysis of condensed gas-off products from the chambers
4. Gas chromatography on a variety of polar and non-polar columns and gas chromatography fractionation with mass spectrometry or infrared identification of collected fractions

Methods are shown in Tables II-V, and figures 2-4.

RESULTS AND DISCUSSION

As anticipated, the major yields of gas-off products occur with the candidate paints and coatings, which desorb entrapped solvents and plasticizers. Lesser, but still significant, amounts of space contaminants result from oxidation, hydrolysis and sublimation processes. Analyses, representing each type, are presented in Tables VI to XI.

Most surprising were the high levels of carbon monoxide, issuing from several candidate materials. In general, the materials do not produce significant amounts of carbon monoxide during the test periods. The several exceptions, as determined in our partial study, are shown in Table VI.

The mechanism by which carbon monoxide is evolved in the chamber from the black, carbon-based coating, cannot be established with certainty from the available data. Two processes are possible, oxidation in the chambers or desorption of previously chemisorbed oxygen. Since a lesser amount of carbon monoxide was detected in the successive 30 + 30-day analysis than in the initial 30-day test, the latter process is believed to predominate, but not to the exclusion of the former. The presence of methane is undoubtedly due to retention of adsorbed methane during the formation of carbon black, which is a major constituent of the coating.

Significant amounts of ammonia and ethyl amine were produced by hydrolysis during the testing of a low molecular weight polyamide (Table VIII). No ammonia or ethyl amine were detected when vapors from fresh polyamide were analyzed by gas chromatography or mass spectrometry.

Preliminary data for materials containing a silicone base indicate the presence of significant amounts of trimethylsilanol and low molecular weight siloxane polymers after the initial 30-day period, but lesser amounts after 60 and 90 days. An apparent condensation to higher molecular weight, less volatile siloxanes occurs. In addition, with materials containing relatively large amounts of ethanol, there is some evidence for oxidation of ethanol to acetaldehyde during storage for the longer periods, i. e., 60 and 90 days (Table IX).

One of the silicone greases exhibited an unusual behavior in the out-gas chamber. Several days after the chambers were charged with the material, fine needle-like crystals were observed growing on the upper inside wall of each chamber. This took place with the air atmosphere as well as with the oxygen atmosphere at 5 psi. The crystals were removed and analyzed by mass spectrometry, proving to be tetrachlorobenzene. Subsequent infrared analysis confirmed the material as 1,2,4,5-tetrachlorobenzene.

No tetrachlorobenzene was detected by analysis of the gas-off products. Apparently, through the sublimation process, the tetrachlorobenzene is deposited in the solid state on the inner surface of the chamber with little, if any, remaining in the gas phase.

Although the deposits are considerable, collection and quantitative measurement of this compound directly from the chamber is impossible due to the random scattering of the crystals. Data was obtained from an auxiliary experiment performed in air at 23°C and 35% relative humidity (Table XI).

Based on preliminary evidence for several dozen candidate materials, in general the differences between the gas-off products produced in the air atmosphere as compared to those evolved in the oxygen environment result mostly from the pressure differences rather than oxidative effects, i. e., more gas-off products are obtained at the reduced pressure. In the early evaluation of the data, there is no evidence for increased oxidation of gas-off products when the candidate materials are stored at 23-25°C under an environment of oxygen at 5 psi rather than air at a pressure of one atmosphere.

CONCLUSIONS:

Some standard procedures for establishing minimum detection levels are needed. The simple methods of weight loss from thermal degradation or olfactory sensing, as used in the Mercury program, will not suffice. As shown in our study, the highly sensitive detection system of gas chromatography and the specific identification possible with supporting mass spectrometry and infrared absorption spectrophotometry fulfill most criteria.

The techniques employed in this program were developed for application to survey a wide range of materials. In each material system, more optimum instrument conditions, particularly gas chromatography operation, could be established. We feel that with improved gas chromatography techniques, considerably lower detection levels can be established.

The biggest problems in standardization of methods are sample preparation and handling. There are many variables, e.g., freshness of sample, surface area, mixing, curing, sample uniformity and changes in proprietary mixes, which can influence the nature and degree of gas-off products.

Our early results indicate that standardization of methods for gas chromatography and mass spectrometry can best be accomplished for individual sample types, e.g., silicone polymers, rather than a single comprehensive approach. Each system produces different gas-off products, which require different analyses. Also, the most significant data can be obtained when the materials are evaluated in the approximate form for final use.

ACKNOWLEDGMENT

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TABLE I
TYPES OF CANDIDATE MATERIALS

Silicone rubbers	Fluorinated greases
Silicone greases	Wax lubricant
Silicone fluids	Dyes
Foam rubber	Silver ink
Polyurethane foams	Polymethylmethacrylate
Adhesives	Polyvinylfluoride
Sealants	Polycarbonate
Primers	Varnish
Paints	Nylon W/molybdenum sulfide
Carbon based coating	Elastic webbing
Fluorinated oils	Adhesive transfer tape

TABLE II

GAS CHROMATOGRAPHY ANALYSIS
FOR CARBON MONOXIDE AND METHANE

Instrument - F&M Model 1609 Flame Ionization Detection
System

Sample Volume - 3.3 ml environmental gas

Analytical Column - 5A Linde Molecular Sieve (60/80 mesh)
(3 ft. x 1/4 in. o.d., stainless steel)
and micro glass beads (60/80 mesh)
(0.5 ft. x 1/4 in. o.d., stainless steel)

Catalyst Column - Ni on 80/100 mesh alumina
(7 in. x 1/8 in. o.d.)

Method - Carbon monoxide is reduced to methane by hydro-
genation at 360°C in presence of a nickel catalyst.
Methane originally present and that produced from
the reduction of carbon monoxide are eluted
separately.

Sensitivity of Detector - 2×10^{-8} grams

TABLE III

GAS CHROMATOGRAPHY ANALYSIS
OF GAS-OFF PRODUCTS IN THE CHAMBER

Instrument - F&M Model 810 Research Gas Chromatograph
with Flame Ionization Detection System

Sample Volume - 25 ml environmental gas

Analytical Column - 5% Carbowax 20M on 60/80 mesh Gas Pack F
(temperature programmed 40^o-230^oC
@ 10^oC/min.)

Approximate Sensitivities - 10⁻⁷ -10⁻⁸ grams

TABLE IV

MASS SPECTROMETRY AND GAS CHROMATOGRAPHY
ANALYSES OF CONDENSED GAS-OFF PRODUCTS

Instruments - C. E. C. 21-103C Mass Spectrometer and F&M
Model 810 Research Gas Chromatograph with
Flame Ionization and Thermal Conductivity
Detectors

Sample Volume - Condensed gas-off products trapped in
25 ml helix

Method - By purging with nitrogen, the gas-off products are
collected at -195°C in a helix having a volume of
25 ml. The composite is first analyzed by mass
spectrometry then partitioned by gas chroma-
tography. Isolated GLC fractions are identified
by mass spectrometry or infrared spectrophotometry.

TABLE V

ABSOLUTE SENSITIVITIES OF GLC INSTRUMENTATION
TO COMPOUNDS FOUND IN GAS-OFF EXPERIMENTS

<u>Compounds</u>	<u>Weight, grams</u>
ethanol	1.4×10^{-7}
isopropanol	4.1×10^{-7}
n-propanol	8.5×10^{-7}
iso-butanol	6.3×10^{-8}
benzene	4.3×10^{-8}
toluene	4.2×10^{-8}
xylene	5.0×10^{-8}
m-dichlorobenzene	8.3×10^{-8}
trichloroethylene	2.5×10^{-7}
methyl methacrylate	4.8×10^{-8}

TABLE VI

CARBON MONOXIDE ANALYSES BY GAS CHROMATOGRAPHY

<u>Candidate Material</u>	<u>Storage Time (Days)</u>	<u>Atmosphere</u>	<u>mg CO per 10 g Candidate Material</u>
Black Coating	30	Air	2.8
	60	"	3.6
	90	"	4.5
	30 + 30	"	0.7
	30 + 30 + 30	"	0.6
	30	Oxygen	4.9
	60	"	4.0
	90	"	5.4
	30 + 30	"	1.2
Varnish	30 + 30 + 30	"	0.9
	30	Air	2.3
	60	"	-
	90	"	2.7
	30 + 30	"	0.3
	30	"	2.0
	60	"	2.6
	90	"	2.8
	30 + 30	"	0.7

TABLE VII

METHANE ANALYSES BY GAS CHROMATOGRAPHY

<u>Candidate Material</u>	<u>Storage Time (Days)</u>	<u>Atmosphere</u>	<u>mg CH₄ per 10 g Candidate Material</u>
Black Coating	30	Air	0.04
	60	"	0.16
	90	"	0.10
	30 + 30	"	N.D.
	30 + 30 + 30	"	N.D.
	30	Oxygen	0.16
	60	"	0.08
	90	"	0.12
	30 + 30	"	N.D.
	30 + 30 + 30	"	N.D.

TABLE VIII
GAS-OFF PRODUCTS - POLYAMIDE

Storage Time (Days)	Atmosphere	Wt. of Component (mg per 10 g Candidate Material)		
		<u>Ammonia</u>	<u>Ethyl Amine</u>	<u>Xylene</u>
30	Air	0.7	0.005	0.001
60	"	1.2	0.006	0.001
90	"	2.6	0.01	0.001
30 + 30	"	2.0	0.004	N.D.
30	Oxygen	1.5	0.006	0.004
60	"	3.0	0.02	0.003
90	"	4.2	0.02	0.009

TABLE IX

GAS-OFF PRODUCTS - SILICONE RUBBER

Storage Time (Days)	Atmosphere	Wt. of Component (mg per 10 g Candidate Material)			
		Ethanol	Acetaldehyde	Silicone Oil	Dichlorobenzene
30	Air	2.1	0.21	0.55	0.005
60	"	1.9	0.44	0.50	"
90	"	1.1	0.66	0.47	"
30	Oxygen	1.1	0.30	0.50	0.005
60	"	1.9	0.53	0.32	"
90	"	0.9	0.67	0.33	"

TABLE X

GAS-OFF PRODUCTS - SILICONE GREASE

Storage Time (Days)	Atmosphere	Wt. of Component (mg per 10 g Candidate Material)			
		Alcohols*	Trimethyl Silanol	Trichloroethylene	Silicone Oil
30	Air	0.04	0.28	0.43	0.29
60	"	0.02	0.04	0.02	0.01
90	"	0.03	0.35	0.04	0.006
30 + 30	"	0.003	0.05	0.02	0.04
30 + 30 + 30	"	0.007	0.01	0.02	0.05
30	Oxygen	0.01	0.45	0.27	0.15
60	"	0.006	0.35	0.06	0.007
90	"	0.007	0.37	0.10	N.D.
30 + 30	"	0.02	0.23	0.17	0.02
30 + 30 + 30	"	0.001	0.001	N.D.	0.001

*Combined ethanol and n-propanol

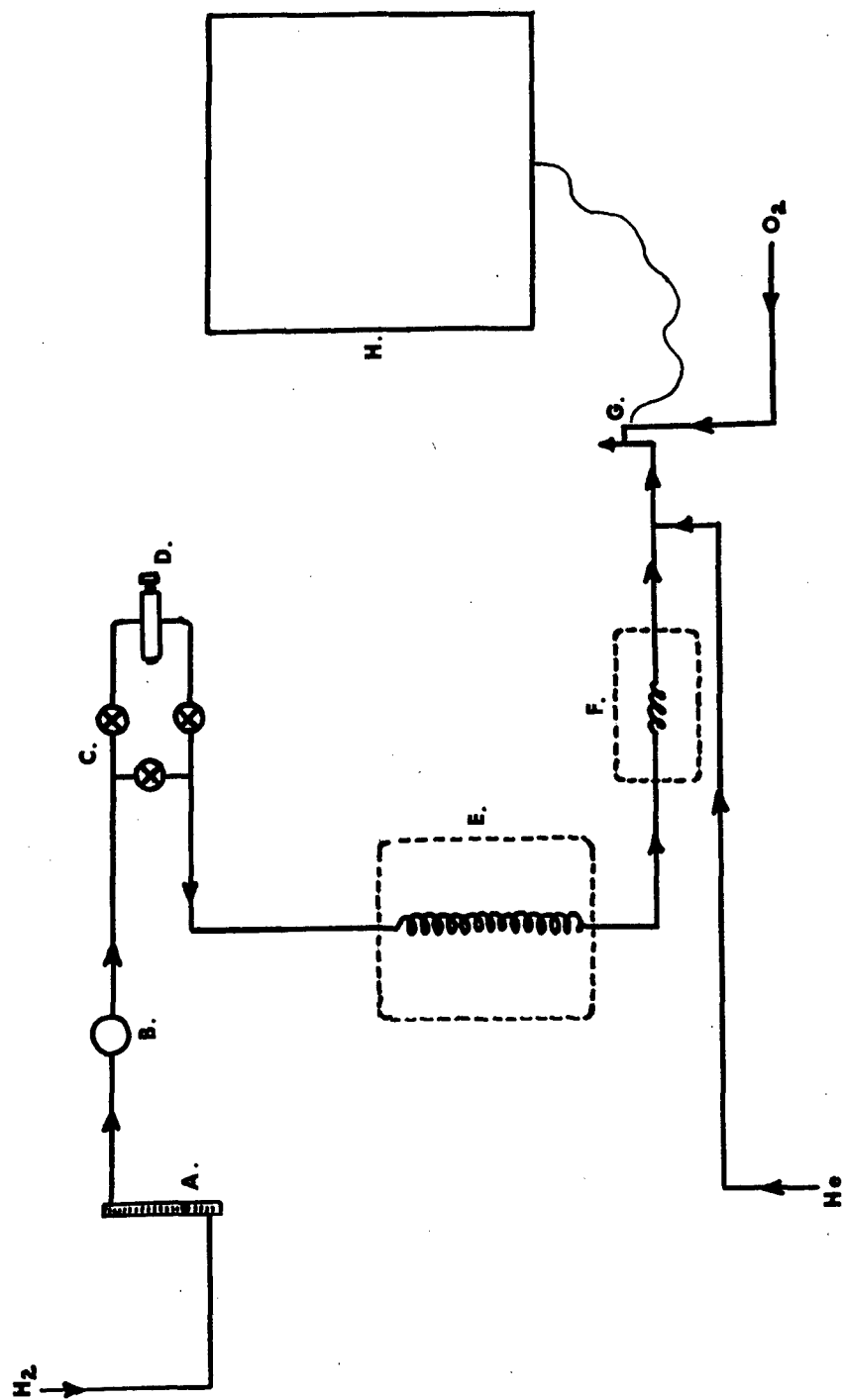
TABLE XI
SUBLIMATE FROM SILICONE GREASE

<u>Storage Time (Days)</u>	<u>Wt. of 1, 2, 4, 5 tetrachlorobenzene (mg per 10 g of Silicone Grease)</u>
30	2.1
60	3.3
90	5.8



FIGURE 1. TYPICAL GAS-OFF CHAMBER AND COLLECTION HELIX

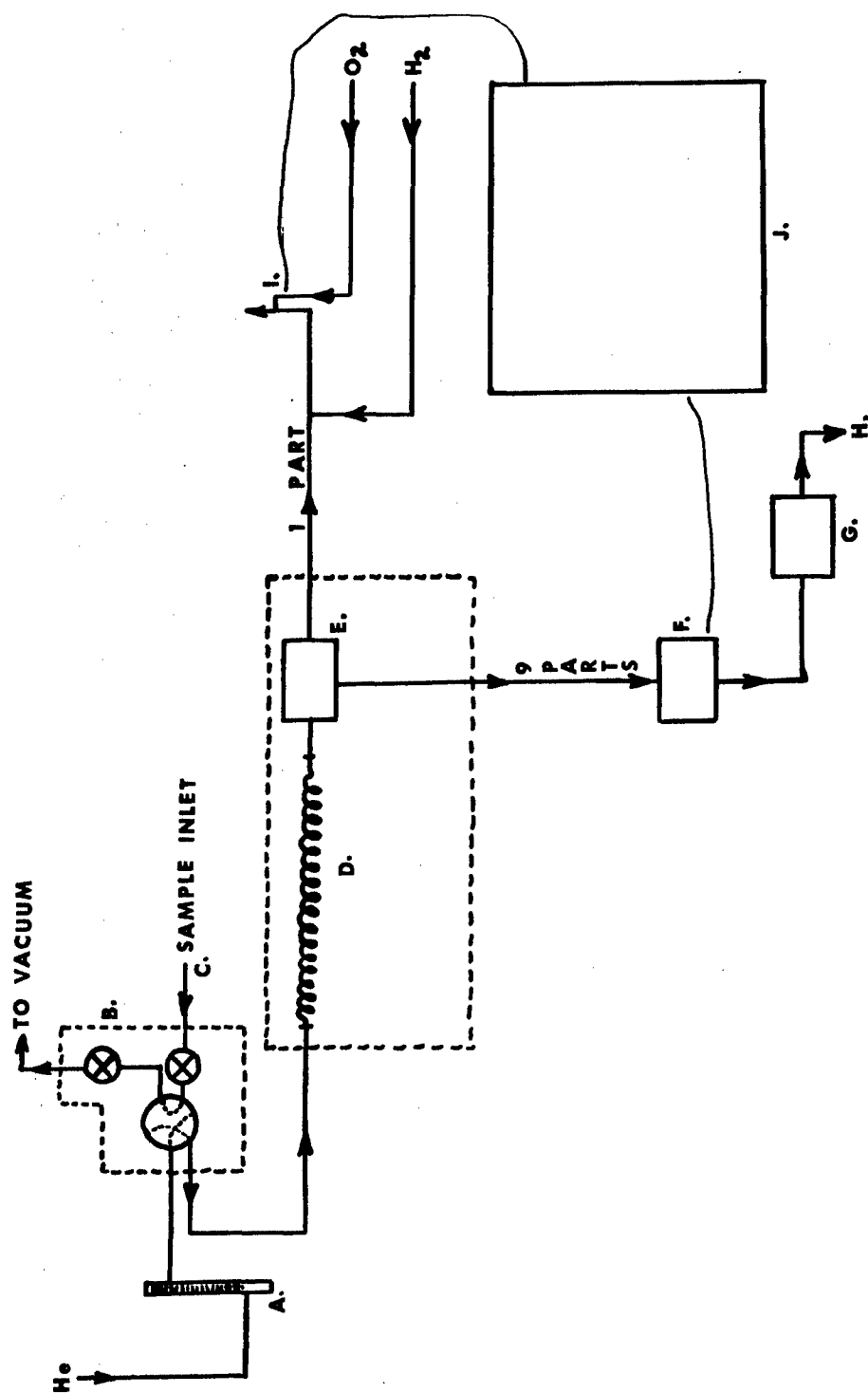
CARBON MONOXIDE AND METHANE ANALYZER



- A. HYDROGEN FLOWMETER.
- B. FLOW REGULATOR.
- C. TOGGLE VALVE BY-PASS SYSTEM.
- D. BARBER-COLMAN GAS SAMPLING VALVE (DETACHABLE).
- E. CHROMATOGRAPHIC COLUMN AND OVEN.
- F. CATALYTIC COLUMN AND OVEN.
- G. FLAME IONIZATION DETECTOR.
- H. AMPLIFICATION AND RECORDING SYSTEM.

FIGURE 2.

F AND M MODEL 810 GAS CHROMATOGRAPH AND SAMPLING SYSTEM



- A. FLOWMETER.
- B. PERKIN-ELMER GAS SAMPLING VALVE WITH TOGGLE VALVES [●] ON SAMPLE INLET AND VACUUM LINE.
- C. GAS-OFF CHAMBER ATTACHES HERE.
- D. CHROMATOGRAPHIC COLUMN AND OVEN.
- E. SAMPLE SPLITTER [1:10 RATIO].
- F. THERMAL CONDUCTIVITY DETECTOR.
- G. FRACTION COLLECTOR AND HEATER.
- H. FRACTION COLLECTOR ATTACHES HERE.
- I. FLAME IONIZATION DETECTOR.
- J. AMPLIFICATION AND RECORDING SYSTEM.

FIGURE 3.

ATMOSPHERIC CONTAMINATION IN SEALAB I

By

Raymond A. Saunders

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Washington, D. C.

The Man-in-Sea Program is one of the Navy's major current research projects. This program has several important goals, one of which is to make possible the free movement of scuba divers for extended periods of time at depths up to 1000 feet. Present practical dives are limited to a few hundred feet for useful work periods measured only in minutes. More importantly, hours of time may be required after such dives to return the divers safely to the surface. Techniques now being investigated by the Navy, however, are designed to permit divers to work at depths of hundreds of feet for 6 to 8 hours a day, day after day, without the necessity for intervening returns to the surface. Such long dives will permit exploration of large areas of the undersea continental shelf, areas which abound in mineral and food riches.

Diving explorations of this magnitude are made possible by eliminating the long decompression periods required for returning divers to the surface. This is accomplished by providing the divers with undersea living accommodations which they may enter or leave at will. After working in the open sea the divers have only to re-enter their undersea home to rest and refresh themselves. They will be able to live comfortably in these quarters for months at a time, secure from their hostile outer environment.

The first U. S. Navy open sea tests of these new concepts were made last summer at Argus Island, a Texas Tower off the coast of Bermuda. The divers' submerged living quarters was called SEALAB I. Figure 1 shows SEALAB just before it was lowered into the ocean. SEALAB's inner dimensions are 10 feet in diameter and 40 feet long. In Figure 2 a diver is standing beside the submerged SEALAB which is resting on the bottom at a depth of 193 feet.

The divers enter or leave the submerged SEALAB through an open hatch in the bottom in the manner shown in Figure 3. Water cannot enter, however, because the atmospheric pressure inside is equal to the water pressure outside. The atmosphere in SEALAB consisted of 4% oxygen, 16% nitrogen and 80% helium at a total pressure of 110 psi. Helium was substituted for most of the nitrogen because nitrogen itself is a toxicant at these pressures.

Interior views of SEALAB taken on the bottom are shown in Figures 4 and 5. Four modern-day Jonahs ate and slept in these submerged quarters for

eleven days. They were provided with a hot plate for heating their meals and, to sweeten their lives even more, they were furnished with a few good dinner wines.

Naturally, one of the many correlative interests in the SEALAB experiments was to determine the nature of the toxicants or contaminants which developed in this exotic atmosphere. A brief description of the methods used at the Naval Research Laboratory for determining the nature of contaminants in such atmospheres is in order.

Previous experience with enclosed atmospheres has shown the impracticality of attempting chromatographic or spectral identification of the many contaminants present in some of these atmospheres by direct, on-the-scene examination of the atmosphere. Various difficulties rule out such a straightforward procedure.

Analysis is most easily carried out by concentrating the contaminants by means of a suitable sampling device and performing the remaining analytical procedures in a suitably-equipped laboratory. An activated charcoal sampler is one practical and convenient method for sampling the more volatile, lower molecular weight contaminants. Such a sampler can be seen in the interior views of SEALAB. In addition to the charcoal sampler, chromatographic and other physical methods of monitoring the atmosphere for the concentration of specific major components were provided top-side. Facilities for the analyses of blood and urine samples were also provided top-side.

The contaminants adsorbed by a charcoal sampler can be recovered from the charcoal by heating it very slowly in an evacuated system. Thermal decomposition of adsorbed contaminants during the desorption process is not a problem if the temperature of the charcoal is raised gradually over a period of several hours to a maximum of 300°C. In this connection it is interesting to note that Messrs. Conkle and Pustinger, who presented the two previous papers, analyzed respectively an environmental chamber atmosphere and the gas-off products from spacecabin materials. Mr. Conkle used a cryogenic sampling technique to concentrate the contaminants from the chamber atmosphere, while Mr. Pustinger's results were derived from direct chromatographic examination without resort to any concentrating procedure. All of the contaminants which were identified in each case, however, had been previously identified at the Naval Research Laboratory in the atmospheres of Mercury spacecraft, atmospheres which were sampled with activated charcoal. In other words, the contaminants reported in the Mercury spacecraft atmospheres, some of which were surprising at the time, really evolved in the spacecabin and were not the product of decomposition reactions during the charcoal desorption process.

The vacuum desorption apparatus used for the SEALAB charcoal is shown in Figure 6. This system is constructed of large bore tubing, valves

and connectors and is entirely greaseless. Traps for retaining the charcoal desorbate are cooled in liquid nitrogen. Contaminants can also be desorbed in this system by elution at any desired temperature with inert gases or with steam. The efficiency with which various contaminants are recovered from charcoal does vary considerably. This fact makes quantitative determinations related to the original atmosphere rather impractical.

Neither is it always practical to qualitatively identify the various contaminants in a charcoal desorbate, or in a sample of whole air for that matter, with chromatographic procedures alone. This is because of the large number of contaminants which may be recovered and the many chemical classes they may represent. One relatively easy and positive method of identifying the individual components of a contaminant mixture, however, is to resolve the mixture with a chromatographic column, trap the components from the column effluent with a suitable fraction collector and identify the components by means of their infrared or mass spectra.

The fraction collector used for this purpose at the Naval Research Laboratory is shown in Figure 7. This fraction collector permits the efficient recovery of any or all of the condensable components of a mixture from the effluent stream of a chromatograph, in spite of the fact that such components are often eluted in rapid succession. This is made possible by a valving arrangement which allows instantaneous switching of the column effluent from one trap to another at the touch of a button and by the unique design of the traps themselves.

Two pint-sized Dewars of liquid nitrogen, used for cooling the collection traps, are placed in spring-loaded holders, one beneath each of two collection stations. The Dewars can be pressed down in their holders to change traps and then released to immerse the traps in the cooling bath. The traps used with this fraction collector are shown in Figure 8. They are Pyrex tubes about the size of a lead pencil, terminated at one end with a rubber serum cap and at the other with a hypodermic needle. In use, a trap is slid onto a collection station and immersed in liquid nitrogen. An organic component is frozen out of the effluent stream onto the walls of the trap. The carrier gas, noncondensable helium, passes through the needle at the bottom and is exhausted into the nitrogen bath. Traps can be replaced at one station while a component is being collected at the other. Used traps are allowed to remain in the liquid nitrogen bath until ready for subsequent spectral examination. No liquid nitrogen can enter the tube during these operations. Moreover, no air can condense in the traps to dilute or contaminate the sample.

It is very easy to transfer collected components from these traps into infrared microcells or into a mass spectrometer inlet system. Very small quantities of material can be collected and identified. Quantities as small as one-five-thousandths of a microliter of liquid, equivalent to

0.5 μ g, have been collected from packed column effluents and spectrally identified. Such quantities give rise to thermal conductivity detector signals from 0.5 per cent to several per cent of full scale at maximum sensitivity. This degree of overall sensitivity means it is possible to trap out of a chromatographic effluent and spectrally identify the more concentrated trace contaminants from a one-liter-sized sample of atmosphere from a submarine or spacecabin. Moreover, the determinations can be quantitative, an accomplishment not practical when charcoal sampling is involved.

A chromatogram of the desorbate mixture recovered from the SEALAB charcoal shows some thirty-four peaks. Mass spectral analyses of the material represented by these peaks indicated in most cases more than two components. The total number of contaminants recovered approximated one hundred. These compounds were mass-spectrally identified or characterized as saturated and unsaturated aliphatic hydrocarbons and aromatic hydrocarbons. The major portion of the desorbate consisted of compounds above C₇ in molecular weight. The overall composition of the mixture was very similar to that of a light hydrocarbon oil such as kerosene. The SEALAB charcoal was nearly saturated with this material. No other types of contaminants were detected. There were undoubtedly other contaminants present, but they were effectively masked by the relatively large concentration of hydrocarbons.

Prior to the actual SEALAB experiment, a three-man, twelve-day chamber test called Project GENESIS was made in New London. Contaminants detected in that atmosphere, for a 24-hour sampling period in the middle of the test, are listed in Table I. The carbon disulfide is believed to have evolved from some foam-rubber products in the chamber.

Table I

Contaminants found in the Project GENESIS atmosphere
Methyl alcohol Ethyl alcohol Acetaldehyde Freon-11 Freon-22 Ethyl formate Carbon disulfide Carbon dioxide

The great profusion of hydrocarbons in the SEALAB atmosphere was unexpected and their source is interesting and important. Some detective work indicated that they evolved from dust filters in a pair of carbon dioxide scrubbers which were operated continuously in SEALAB for the eleven days submergence. The history of these scrubbers is also interesting in light of subsequent events.

In the days before nuclear submarines, the submergence capabilities of conventional submarines was determined by the buildup of carbon dioxide in the atmosphere. Operation of a relatively simple carbon dioxide scrubber enabled the submarine to remain submerged for an additional period up to the limit of the ship's battery capacity. These CO₂ scrubbers employed LiOH, a dusty material which is very irritating to the respiratory system. To keep this dust out of the ship's atmosphere a paper filter was used which had originally been developed for filtering dirt particles from light hydrocarbon fuels and hydraulic fluids. The manufacturer of these filters immersed each unit under air pressure in a light hydrocarbon oil to test for flaws in the paper or leaks in the seals. The oil retained in the pores of the filter paper posed no problem to its intended use, nor for the Navy's application as a dust filter. In fact, its effectiveness as a dust filter was enhanced. Such CO₂ scrubbers are not used on nuclear submarines, although two or three are carried aboard for emergency situations.

Being conveniently at hand in the Navy supply system, however, two of these units were used in SEALAB and effectively controlled the carbon dioxide concentration. The hydrocarbons which volatilized from these filters into SEALAB's atmosphere were relatively innocuous compounds and were no particular threat to the occupants. Neither were these filters or their hydrocarbon content a fire hazard since the low oxygen concentration of SEALAB's atmosphere could not have supported even momentary combustion.

Just recently, however, a fatal accident attributable to the use of these filters occurred in Washington. Two divers under pressure in a low-oxygen atmosphere were transferred to a decompression chamber in which one of these CO₂ scrubber units was operating. Decompression required a high-pressure, enriched oxygen atmosphere. Soon after the men entered the chamber, ignition was reported to have occurred in one of the filter cartridges. Within seconds the entire chamber interior was a ball of flame. This is another tragic reminder, if one be needed, of the constant vigilance necessary with high-oxygen atmospheres.

The techniques just described for identifying contaminants in closed atmospheres work extremely well when dealing with the relatively clean atmospheres found in spacecraft and environmental test chambers. Such atmospheres usually harbor less than fifty detectable contaminants.

However, a nuclear submarine atmosphere, the granddaddy of all closed atmospheres, contains several hundred contaminants. These procedures become much too time-consuming for a detailed analysis of this magnitude.

In order to study the detailed composition of the submarine atmosphere the Naval Research Laboratory has purchased a high-resolution, rapid-scanning, analytical mass spectrometer. This instrument has a wide mass range, a double ionizing source, and very high sensitivity. The new spectrometer will be used to monitor directly the effluent of high-resolution, temperature-programmed, capillary columns. One of the ionizing sources of the mass spectrometer will serve as the chromatographic detector. The effluent will also be monitored by an automatic peak area integrator and print-out system. In addition, the integrator unit will automatically trigger scans of the mass spectrometer at appropriate points. This analytical system will permit direct qualitative and quantitative analyses of the more concentrated contaminants in submarine air without recourse to charcoal sampling. Moreover, the time required will be much less than that now required to provide only qualitative identifications of much simpler mixtures.



FIGURE 1. SEALAB TOPSIDE IN PREPARATION FOR SUBMERGENCE



FIGURE 2. DIVER AND SEALAB ON THE BOTTOM

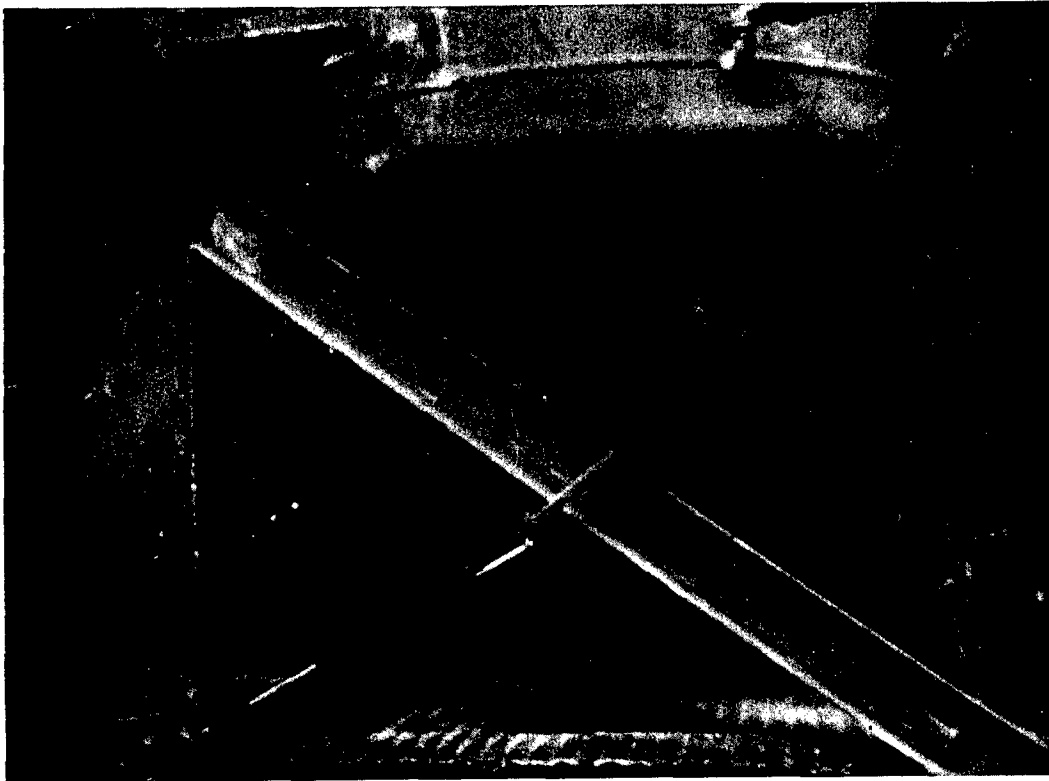


FIGURE 3. METHOD OF ENTERING SEALAB THROUGH AN OPEN HATCH

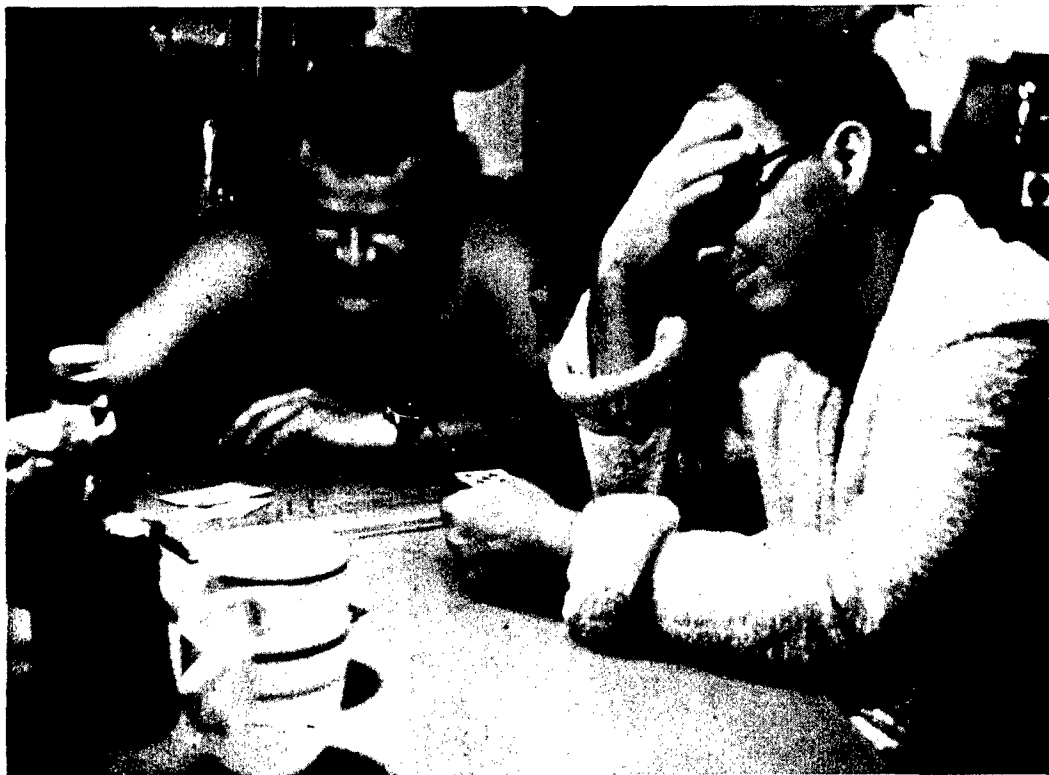


FIGURE 4. INTERIOR VIEW OF SEALAB (Air Sampler Immediately to Right of Fan)

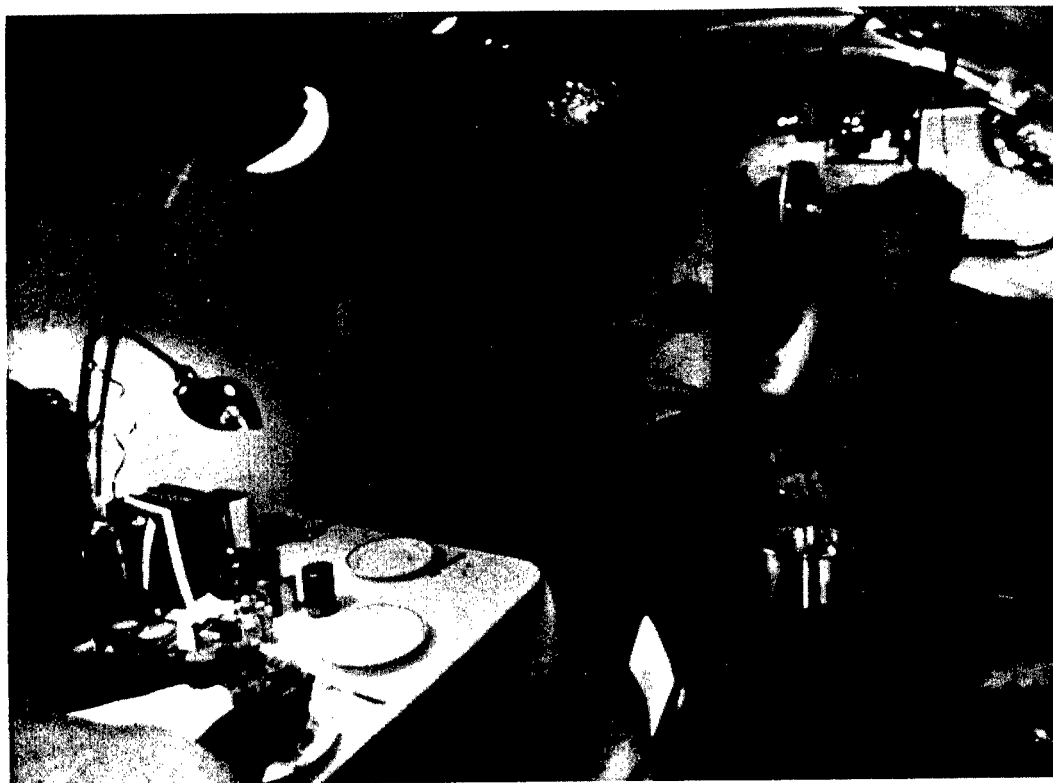


FIGURE 5. INTERIOR VIEW OF SEALAB

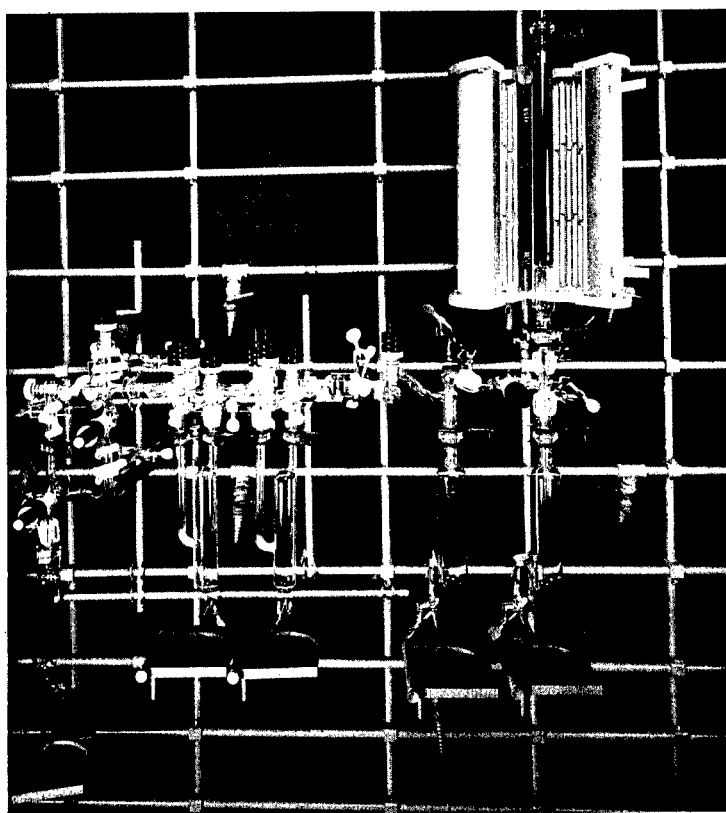


FIGURE 6. CHARCOAL DESORPTION APPARATUS

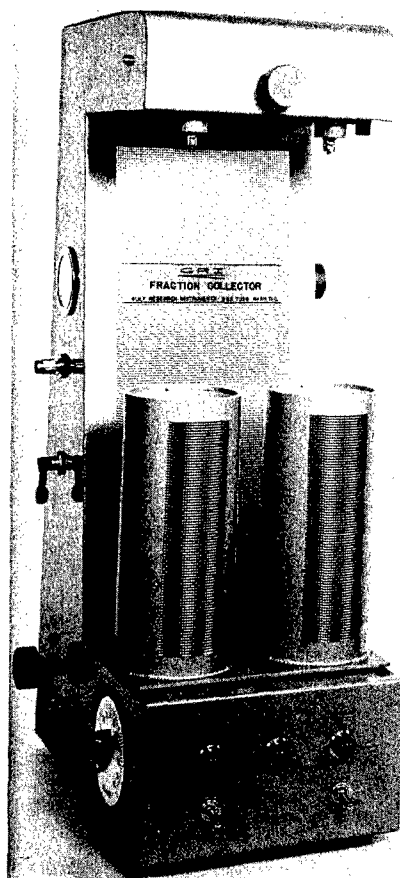


FIGURE 7. FRACTION COLLECTOR

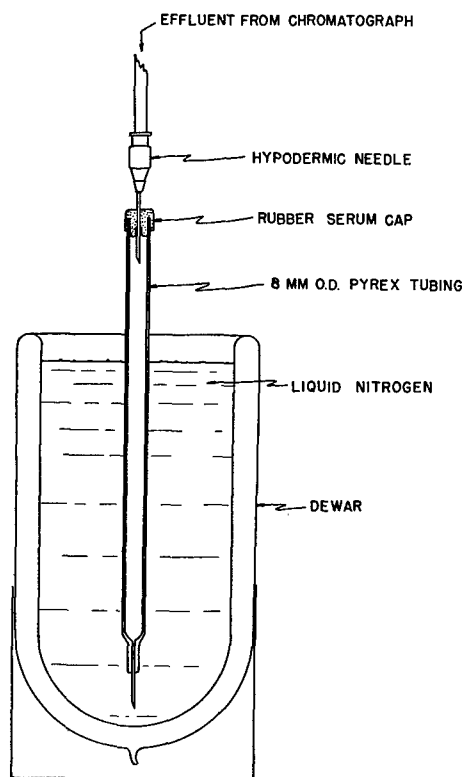


FIGURE 8. COLLECTION TRAP

QUESTION AND ANSWER SESSION

MR. WILSON: (Boeing) I would like to ask a question of the first speaker regarding whether or not it has been demonstrated that no chemical changes or decomposition takes place by collecting a sample at a temperature of -195° .

MR. PUSTINGER: Obviously, you have potential oxidation of components. In our case, we had an air atmosphere and you condense it at -195° . This is one reason why we were doing most of our analyses based on sampling directly from the gas-off chamber. We don't have enough data to say there is or is not some oxidation going on or hydrolysis, or whatever the case may be.

MR. WILSON: You've not made comparisons?

MR. PUSTINGER: Well, we have, but it isn't in sufficient form that I can make a comment on that.

DR. HARRIS: One of the things I wanted to question you about was the similarity in most of the results between 5 PSI oxygen and air. The results you presented were quite similar, in many cases.

MR. PUSTINGER: That is correct. That's one reason we felt most slight differences were due to gas-off at the slightly different pressures rather than any oxidation effects or things of this nature.

MR. WILLARD: (Honeywell) This is a question I want to pursue a little bit further. There has been a good deal of discussion among those of us doing gassing studies on the effect of total pressure on gassing characteristics. Some relatively limited work that we did at Honeywell indicated that the difference was primarily attributable to oxygen concentration or oxygen partial pressure, if you will, rather than total pressure; so the results that we have would indicate just the opposite conclusion that you stated, that the differences were attributable primarily to total pressure difference. Could you give us a little bit of insight into the facts which led you to your conclusion, that the differences were attributable primarily to total pressure?

MR. PUSTINGER: Well, the facts, as best I can say, would be the comparison of the gas-off materials as we observed it on the slide. Again, I repeat, we did not do definitive studies on all the samples, but we did notice that the materials that presumably would be oxidatively stable were increasingly present in the lower pressure systems. Other than that, I can't make any more comments.

DR. HARRIS: Mr. Holden, you've done some work in that area, haven't you?

MR. HOLDEN: No, we just did 5 PSI straight 100% oxygen. We haven't varied the pressure level.

DR. HARRIS: I see. Are there any other questions?

COL. DILLS: (SAC Headquarters) I would like to ask Mr. Saunders if he could give us a feel for what contaminants are there that are built into the system as opposed to the people who are working in the system or what they might carry in with them, shaving lotion, etc.?

MR. SAUNDERS: I think they try, in space cabin environmental control chambers, to avoid these materials that people would carry in with them. On a submarine, of course, they don't. So you can find any of those materials on a submarine. I think, in fact, that you can find anything in a submarine. I mentioned earlier, I think, that most of the detectable components we find in these atmospheres evolve from materials rather than people themselves. I don't doubt that there are contaminants in these atmospheres which originate with the personnel, but they are at low levels of concentration, lower than the others, perhaps lower than our present limits of detection.

MR. WILLARD: (Honeywell) Without wishing to belabor a point again, Mr. Pustinger, on the difference of gas-off products between air and 5 PSI oxygen, did you run confirmatory studies at the same oxygen pressure but different total pressure? The oxygen partial pressure in air, of course, would be lower than the oxygen pressure at 5 PSIA, and, therefore, it could appear from what you have said so far that the difference would be attributable equally to increased oxygen pressure or to decreased total pressure.

MR. PUSTINGER: I'm afraid I'm not going to be able to answer your question too well. All I can say is, again I repeat, that we did not do intense studies on these materials. We are testing them just at one atmosphere in air and 5 PSI, pure oxygen.

DR. HODGE: In the past two days, we've heard a lot of very fine presentations about the characterization of the extremely intricate mixture of substances in trace amounts in the confined atmospheres. We've also heard a number of papers on studies of the effects of some of these materials on experimental animals. I'd like to follow Dr. Stokinger's point of view yesterday and ask about the plans for the control studies on man? I can think of two or three ways that this might be approached, and perhaps these are all outlined. One question, having obtained some information on animals, since man is the object of our concern, control studies of exposure to a single component at ambient pressures and 5 PSI, another rather obvious question -- can the man be put in the space capsule for the period of time or longer that he is expected to reside in actual flight, and have analyses not only made, as he will, but, with the facilities of the laboratory around him, a really much more thorough analysis?

DR. HARRIS: Dr. Hodge, you're getting on a rather touchy point. I'm not free to discuss whether one can put a man into the chamber and consider him as an experimental subject during the course of a run. That's all that I can say at the present time.

Have you any further questions?

DR. THOMAS: May I say a word? I think we, on the Air Force's part, will probably do it, if we get stuck with a situation where extrapolation from animal to human is unsafe; that is, if you see a great species difference in response -- then the ultimate test is volunteers, there's nothing else you can do. We are doing this every day, not in toxicology now, but we do it with heat chambers, we do it with centrifuges, there's nothing wrong with that, that I can see.

DR. CULVER: (Aerojet-General) I'd like to go back to your paper, Dr. Harris, of this morning and ask -- what is the justification, validity or importance of the olfactory test? It cannot be really -- odor cannot be related to toxicity. We know that in other confined atmospheres, in submarines, they're pretty stinky buckets. Why do we use this as the first gate or screen by which you drop the materials?

DR. SCHWARTZ: Odor can be pretty disabling. I want to quote something -- recently where they used a non-toxic nauseating compound in Vietnam it was very effective, completely non-toxic, produced a nauseating odor and was completely disabling. Why should we put something in there that we know is going to be nauseating and/or irritating? (Another example, of course, is tear gas) -- When this could be eliminated with perhaps the simplest test, the odor panel.

DR. CULVER: I consider nausea and irritation to be toxic manifestations. I'm talking about odor only and where you cannot relate odor to toxicity, why use the test? I would think that our spacecraft engineers should work in a paper factory for a while before they decide that odor is such an important factor in performance.

MR. WILLARD: (Honeywell) I'd like to comment on this from the viewpoint of Honeywell, and I think I can speak for Mr. Welhart of McDonnell also. As you probably know by now, all of the testing of materials for their potential toxic hazards on Mercury and Gemini were done on the basis of odor testing. It does have one tremendous advantage. It is fast. It is cheap. You can run through a lot of materials in a short period of time, and I think the results of the analytical studies on Mercury and Gemini atmospheres so far pretty well bear out that it is a good tool with which one can do a lot of effective work. It certainly is not the only tool; and, for long-term exposure, our viewpoint at Honeywell is that it is not a totally valid tool. The way in which we use odor tests is as a screening device. We can throw out a lot of

objectionable materials, which we can readily replace. We can do this on the basis of a very fast and inexpensive test. If we haven't got a ready replacement for the material, if we really need that material, we then go on to the more expensive and more time-consuming tests, chromatographic analyses and so forth. This, then, is the prime justification in my own mind for odor testing. It is a convenient, fast and inexpensive tool for discarding materials that are replaceable with others that do not exhibit characteristic odors.

DR. HARRIS: Is there any reply to that?

FROM THE FLOOR: I'd just like to add something to that. One of the silicone compounds we tested for outgassing, we isolated and identified acetic acid at a very low level, approximately 12 micromoles per 100 gram sample. Now, looking at this from an outgassing rate, it is quite low, yet from an odor point of view, it was totally rejected as quite irritating.

DR. ROTH: (Lovelace Foundation) I would like to ask whether animals given these same diets as humans would produce essentially the same gaseous products?

DR. HARRIS: It depends on intestinal flora. As a matter of fact, if you've ever had a boxer dog around, eating table scraps, I can assure you that the odor is distinctly different. Quantitatively, I can't say what the differences are. Qualitatively, yes. The intestinal flora are different, aside from differences in various facets of metabolism. Mr. Conkle made mention of the fact that they were going to do, or had been doing, some outgassing of animals in the chamber. I think perhaps he might have some comments on that.

(Mr. Conkle had already departed.)

DR. DU BOIS: Mr. Chairman, I'd like to return to the delicate subject you don't want to discuss, about man and exposing him to the capsule, and throw in the viewpoint of the physiologist, and some of the factors that probably would make this a safe and reasonable thing to do. In medicine, we have to deal with these things quite frequently. Now the toxicological end point of, say, mortality, isn't useful in man; but the physiological test may show significant change in the individual if it's a change of 10% in the function, say the function of the liver, the function of the lungs, the function of the heart. You can pick up a significant change at the 10% level. The level at which functional change may be fatal may be at the 1000% level. That is, the lungs have a reserve of tenfold, this is to accommodate exercise. This is in diffusing capacity, airway resistance, lung compliance. Similarly, the liver and other organs have ranges in their functional reserve of perhaps 1000%. Now, therefore, if under controlled conditions you expose a man to a situation in which he may develop a 10% change that you can detect, and if you see it's going past the 20 or 30% level and stop the experiment, you are reasonably safe, because

very often these are reversible processes; particularly if you know what the process is, because you still have a wide range of safety before you get to anywhere that he would proceed at decremented function or approach a mortality. Now, there are two other safety factors. Man could be preceded by a hen, a dog or a pig, as he was in the first hot-air balloon sent from Versailles, as shown in the Air Force Museum. So, these could precede him in the capsule. He could be accompanied by a canary or a mouse, as men have been on entering mines. These are animals that show the same response, but show it in a much shorter time span because the metabolic process is approximately three times as fast as that of the man. I don't think we should sweep such delicate questions under the rug. I think we ought to bring them out and talk about them.

CLOSING REMARKS

Dr. George Kitzes, Conference Chairman

I think this matter of using man in the system is a matter of semantics. Was it Dr. Hodge who put on the board, yesterday, the word "Vocabulary"? Today man is being used as an experimental subject, but we call these experiments "simulators". At some point in time, when you have obtained the maximum data that you can from the laboratory by the use of animals, you go to man. Take your threshold limit value -- your best judgment is used to select a tentative value with a built-in safety factor. Then man becomes the experimental subject. And you may or may not revise the level, the threshold limit value, up or down. So it is a matter of philosophy and definition; but, for the purposes of avoiding the publicity attached, to those who do not know the proper vocabulary and interpretation, we do not plan to use man as an experimental subject. We ask him to go into a simulator.

We have an excellent life support simulator -- we call it an "evaluator". We move into the evaluator everything that he's going to live with. We try to duplicate everything that he will encounter. But this doesn't mean that we are going to expose him to a known toxicant. I think Dr. Hodge's point is that we're going to reach a point of diminishing returns. When you obtain the tremendous amount of knowledge and in terms of the total number of materials that he will encounter, you have to make a judgment. A judgment can be, again, defined as calculated risk. Make a judgment to the best of your knowledge. After that, we can obtain the more finite data by using man, which is the ultimate objective.

With that, I'd like to go on and I'd like to leave unanswered questions. I like to feel that when we leave we have many problems that have to be solved so that we will have occasion to call you together again at another time in the future. We realize that we have reached a new era here in the field of toxicology; that, although much of the discussion yesterday indicated that classical toxicology may not be suitable for our applied problems in the Air Force, NASA or the Navy, it doesn't mean that we can't use classical toxicology. We just have to supplement that which is known with future techniques. For instance, yesterday the panel mentioned that it would be highly desired to have unbroken exposure, so-called "continuous" exposure. Today we can say that we can supply this to the toxicologist. Those who had the good fortune of visiting our facility could see that with the use of the Thomas Domes we can provide continuous exposure. Yesterday the panel mentioned the need for a continuous analysis based on experience of those who have done much in inhalation toxicology. Again we have learned from the papers that were presented in the past two and a half days that there are many advances in the techniques for the analysis of small amounts of materials. Continuous analysis. The use of computerization, the use of the gas chromatograph, and all the other new techniques.

Some discussion was devoted to the importance of methodology and accuracy. This has not changed. When I was taking a course in physical chemistry, it was stressed at that time. There is a difference between precision and accuracy. This we all recognize, and there's no question, and I don't think there's much need for a group like this to discuss the value of precision and accuracy and the difference. These should be taken into account, not only in the analysis of the air for the contaminants, but also in clinical chemistry. For those who can't appreciate the importance of accuracy I hope are not attending this meeting or do not have any place in toxicology or any scientific research. We can just set this aside saying that this is a known fact, that the best methods, control procedures and techniques will be used to obtain uniformity of results. When we get to the selection of test animals, unfortunately, we haven't made too much progress. We have enjoyed the description of the frustrations of our research scientists in the past who would attain an ideal test animal -- in fact, would you say that man is an ideal test animal? Heavens no! We have selected subjects for our simulators, taking into account that before we do, he requires a physical examination, including a medical examination, plus a psychological screening to make sure he is compatible with the other subjects. This, in itself, could produce a stress, and then, lo and behold, we found that we'd missed something. We forgot to look at his teeth. We almost aborted an experiment because there was a flareup of a bad tooth, in fact, part of it broke off; so, in recognition of this, we had to include a dental examination, as complete as possible, so as to eliminate that factor. So, is man an ideal animal? The answer is "no", but he's the best we have.

Coming back to animals -- strictly because of the philosophy that we have in our society, which we can discuss for many hours; however, we will use animals, because we do appreciate the safety problems and we certainly don't want to injure any of our subjects. So there's a need for animal experiments. I think the conclusion from this meeting and from the panel is that we still have to count on human judgment. I'd like to stress that again and again, because this is the theme song for the Air Force. We need man in space. There would be some who would feel that there is no place for man in space because they have an instrument or machine or black box to do everything that has to be done. They can't do everything that man can do. So, it's a matter of judgment. I think today we have to recognize in the selection of test animals that there is this judgment which is based on training, experience, insight and the unknown, which some have and are born with. We will recognize the disadvantage of using certain animals and continue to proceed to use the best combination to give us the most useful results.

Coming to the next factor, which I think is going to play an important role in our program. I hope others who feel they have something to contribute to the overall problem of air pollution in space cabins, confined spaces, contribute to the establishment of a Clean Air Act for space, flying spaces, in terms of the attention that should be paid to the individual animal subject. I was very much impressed by this approach and glad that a number of you agree that we

can capitalize on the knowledge that we have, and the techniques that we have, not only in the interests of economy, but in obtaining the precise information concerning the effects of one or more contaminants, multiple contaminants, on the animal. I think it was concurred in by the panel yesterday, that we should enlarge the scope of our study on the individual animal. I believe that, speaking for Dr. Thomas and myself, this will become part of our program, especially when we get to the use of larger animals, monkeys and chimps.

As far as making it clear that we are going to continue to use established methods, it has been made very clear, and I have been asked to notify the group here that we have no intention of disregarding well established methods of clinical chemistry, hematology and histopathology. We feel that we would be remiss if we ignored these necessary examinations. I can give you an example whereby it would be sheer negligence if we overlooked the value of histopathology. Equal to the experience of many of you in the audience who have had the privilege of using the new technique of electron-microscopy, we had occasion to show that exposure to 100% oxygen, which is essentially 100% oxygen at ambient pressures, is lethal to animals and, we feel, lethal to man, although we have not proven it using man as a subject; but we know definitely that it is lethal to rats and dogs in a relatively short period of time -- 64 to 72 hours.

However, the strange thing is that the histopathological examinations of tissues like liver and kidneys using light microscopy did not indicate any abnormality. However, the use of the electron microscope showed definite cellular and sub-cellular changes, including the mitochondria. Continuing this approach, we found that under other conditions of exposure, 100% oxygen, and this was at 5 PSI, these same changes occurred, which later resolved themselves, indicating that there is a necessity to continue the study, the histopathological study, of our animals, even though they do not show overt changes, or overt effects; so we will include this as part of our minimal criteria, the gross and histopathological examinations of our animal subjects, using again our clinical, scientific judgments of what to do and how much to do.

BACKGROUND NOTES ON THE PRINCIPAL SPEAKERS

BACK, Kenneth C.

Dr. Kenneth C. Back received his Bachelor's Degree in Natural Sciences from Muhlenberg College, Allentown, Pennsylvania, and obtained his Master's and Doctoral Degrees in Pharmacology from the University of Oklahoma. He had had industrial experience in pharmacology before his assignment in 1960 to the Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, as a pharmacologist-toxicologist.

CONKLE, James P.

Mr. James P. Conkle attended the University of Maryland and Trinity University in San Antonio, Texas, where he received his Bachelor of Science Degree and Master's Degree. He has worked in the areas of environmental control and analysis of fluids and gases. Mr. Conkle is a physical chemist in the Environmental Systems Branch, USAF School of Aerospace Medicine at Brooks Air Force Base, Texas.

HARRIS, Elliott

Dr. Elliott Harris is a graduate of the Universities of Colorado and Southern California. He worked at the Wyatt Institute of Medical Research at General Electric, doing research on cancer and biochemistry, and in 1962, he began working for the National Aeronautics and Space Administration, Manned Spacecraft Center at Houston, Texas, in the Space Medicine Branch of the Crew Systems Division. He is the NASA counterpart in the area of toxicology of space cabins to Dr. Thomas for the Air Force. Dr. Harris is responsible for the areas of biochemical and toxicological problems associated with cabin environments and crew support.

HAYS, Harry W.

Dr. Harry W. Hays graduated from Franklin and Marshall College and received his Master's Degree and Doctoral Degree in Biology from Princeton. He has been a resident associate at Princeton, a biology instructor, resident

pharmacologist with Ciba Pharmaceutical Products, Inc., and associate professor of pharmacology at Wayne State University. Since 1957 he has been Director of the Advisory Center on Toxicology of the National Research Council. Dr. Hays is the current President of the Society of Toxicology.

HUETER, F. Gordon

Dr. F. Gordon Hueter received his Bachelor of Science, Master's and Doctoral Degrees from the University of Maryland. He received the Borden Agricultural Scholarship Award, taught at Oregon State University and now is Chief of the Physiology Section in the Laboratory of Medical and Biological Sciences, Division of Air Pollution, at the Public Health Service in Cincinnati, Ohio.

INNES, James R.

Dr. James R. Innes was educated in Scotland and England and received his Ph.D. from Cambridge in 1932. He has held many positions of note, including positions with the National Institute of Health, National Multiple Sclerosis Society, U. S. Army Chemical Center, Wellcome Research Laboratories, Brookhaven National Laboratories, all in the field of Pathology or Neuropathology. He is presently with Bionetics Research Laboratories at Falls Church, Virginia.

KITZES, George

Dr. George Kitzes received his Master's Degree and Doctoral Degree in Chemistry from the University of Wisconsin. He worked as a research biochemist with White Laboratories and then for the Veterans Administration. In 1951 he was assigned to the Aerospace Medical Research Laboratories at Wright-Patterson Air Force Base, working in the field of industrial toxicology. He is presently Chief of the Physiology Division.

LAWRENCE, Pope A.

Mr. Pope A. Lawrence is a commissioned officer in the regular corps of the U. S. Public

BACKGROUND NOTES ON THE PRINCIPAL SPEAKERS (cont'd)

Health Service with the grade of Scientist Director, Captain. Mr. Lawrence has a Master's Degree in Chemical Engineering from the Massachusetts Institute of Technology and a Master's Degree in Industrial Hygiene Engineering from Harvard University. Prior to World War II he was a chemical engineer in private industry, and in 1942 was commissioned in the U. S. Public Health Service, where for 10 years he served on a loan basis as head of industrial hygiene programs for the state health departments of Texas, New Mexico and Utah. The following 10 years were spent at the National Cancer Institute in Bethesda, where he was responsible for a variety of environmental cancer field studies, as well as a long range study on the health status of American uranium miners. During the last 3 years, Mr. Lawrence has had assignments in the Office of the Surgeon General; and, after the Clean Air Act of 1963 was passed, he was assigned as Chief of the Federal Agencies Section of the new Abatement Branch of the Division of Air Pollution, Bureau of State Services of the Public Health Service.

MAC EWEN, James D.

Dr. James D. MacEwen received his Bachelor's Degree in Chemistry from Wayne State University in 1949, his Master's in Public Health from the University of Michigan in 1957 and his Ph.D. in Physiology and Pharmacology from Wayne State University in 1962. He has held numerous posts in the field of toxicology and industrial hygiene including an assistant professorship at Wayne State University in the Department of Industrial Medicine. Dr. MacEwen is at present Director of Aerojet-General's Toxic Hazards Research Unit at Wright-Patterson Air Force Base.

MC NERNEY, James M.

Mr. James M. McNerney received his Bachelor of Science Degree in Zoology and Chemistry, his Master's Degree in Biology and his Master of Public Health Degree in Occupational Health from the University of Pittsburgh. He was a Fellow at Mellon

Institute, where he also was Chief Toxicologist for the Industrial Hygiene Foundation. He now holds the position of Associate Director of the Toxic Hazards Research Unit of Aerojet-General Corporation at Wright-Patterson Air Force Base, Ohio.

PINKERTON, Mildred

Mrs. Mildred Pinkerton obtained her education at the University of Rochester and St. Luke's Hospital, Bethlehem, Pennsylvania. She obtained her certification as Medical Technologist in 1943 and since has had numerous positions involving many different areas of laboratory medicine, but particularly clinical chemistry. At present she is Research Medical Technologist in the Toxic Hazards Branch, Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base.

PUSTINGER, John V.

Mr. John V. Pustinger received his Bachelor of Science Degree in Chemistry from the University of Dayton, and his Master's Degree in Chemistry from Pennsylvania State University. He has a wide area of work experience involving X-ray diffraction studies, emission spectrographic experience, nuclear magnetic resonance, ultraviolet analysis, infrared analysis and infrared spectro structural correlations. Mr. Pustinger, an analytical chemist, is associated with Monsanto Research Corporation in Dayton, Ohio.

REEVES, Johnie L.

Lt. Colonel Johnie L. Reeves, VC, USAF, obtained his Doctor of Veterinary Medicine Degree from Texas A. & M. in 1950, his Master's in Physiology from the University of Minnesota in 1957 and his Ph.D. in Physiology from the same institution in 1961. He has served in the United States Air Force Veterinary Corps since 1950 in many capacities. During this time, he has done considerable research on physiological and biochemical mechanisms. Lt. Colonel Reeves is Chief of the Physiological Chemistry Branch of the United States Air Force School of Aerospace Medicine at Brooks Air Force Base, Texas.

BACKGROUND NOTES ON THE PRINCIPAL SPEAKERS (cont'd)

REYNOLDS, Herbert H.

Major Herbert H. Reynolds holds Bachelor of Science, Master of Science and Ph.D. Degrees in Experimental and Clinical Psychology. He has been in the Air Force for 16 years and worked in the behavioral sciences for the majority of that time. Major Reynolds is certified as a psychologist in New Mexico and currently holds an appointment as Consulting Professor of Psychology, University of New Mexico, and as Associate Professor in the Graduate School of Baylor University. He is Chief of the Comparative Psychology Division and Deputy Commander of the Aeromedical Research Laboratory at Holloman Air Force Base, New Mexico.

ROUNDY, Robert W.

Mr. Robert W. Roundy attended Clarkson Memorial College of Technology at Potsdam, New York, and graduated in 1945 with a Degree of Bachelor of Chemical Engineering. Since 1946 Mr. Roundy has been with Aerospace Medical Research Laboratories at Wright-Patterson Air Force Base, Ohio, as an engineer with experience in research and development work associated with aircraft, liquid oxygen containers and oxygen systems. Mr. Roundy is Chief of the Biotechnology Division, where applied research concerning life support equipment is carried out.

RUSTAGI, J. S.

Dr. J. S. Rustagi received his Bachelor and Master's Degrees in Mathematics from the University of Delhi and his Ph.D. in Statistics from Stanford University. He has taught in several universities in this country and is currently an Associate Professor of Mathematics at Ohio State University, Columbus, Ohio. He is the principal investigator for a Public Health Service Project on the study of biological storage systems.

SAUNDERS, Raymond A.

Mr. Raymond A. Saunders has been associated with the Naval Research Laboratory

in Washington, D.C. since 1946. He received his Degree in Chemistry and Physics from Northeastern University in Boston, Massachusetts. He was responsible for the detection and identification of all contaminants which developed in the circuit and cabin atmospheres of United States orbited spacecraft. Over 60 contaminants were identified in these atmospheres. Mr. Saunders is a consultant to the National Aeronautics and Space Administration on problems involving atmospheric sampling and analysis and to Inter-Gulf Research Instrument Company on instrumentation used in air sampling and analysis.

SIEGEL, Jac

Captain Jac Siegel, MSC, USN, took his undergraduate work in Chemistry at New York University and his graduate work in Chemistry and Chemical Engineering at the Brooklyn Polytechnic Institute and at Columbia University, New York. Upon the establishment of the U. S. Navy Toxicology Unit in 1959, Captain Siegel was appointed as the first Officer in Charge. This facility has grown and become one of the most productive facilities in toxicology in the country.

SPENCER, Howard C.

Dr. Howard C. Spencer attended the College of Emporia, Kansas, where he obtained his Bachelor of Arts in 1931 and received his Master's and Ph.D. in Biochemistry and Pathology from the University of Nebraska in 1935. He then taught Biochemistry at the University of Nebraska in Omaha for two years before coming to the Biochemical Research Laboratory of the Dow Chemical Company in 1937. Dr. Spencer has been active in various phases of toxicological evaluation, particularly in the field of food additives. During the last two years, he has been particularly interested in this latter field because of his work with beryllium compounds.

BACKGROUND NOTES ON THE PRINCIPAL SPEAKERS (cont'd)

THOMAS, Anthony A.

Dr. Anthony A. Thomas received his Doctorate of Medicine from the University of Budapest in 1944. From 1944 until 1951 he practiced internal and industrial medicine in Hungary, leaving the country as a refugee in 1951. From 1951 to 1955 he worked for the United States Army as a Clinical Pathologist in Austria and West Germany, emigrating to the United States in 1956 to accept a post as a research toxicologist at the Aerospace Laboratories, Wright-Patterson Air Force Base. Dr. Thomas became a citizen in 1962. Since 1958 he has been Chief of the Toxic Hazards Branch, Wright-Patterson Air Force Base, Ohio. Dr. Thomas has been active as a USAF liaison member of the Committee on Toxicology, National Research Council, has served on the Tri-Service Steering Committee on Toxicological Research of the Advanced Research Projects Agency and currently holds an Associate Professorship as a visiting lecturer in Toxicology at Ohio State University. Dr. Thomas has published numerous scientific papers on propellant and aerospace toxicology and is a Charter Member of the Society of Toxicology.

VERNOT, Edmond H.

Mr. Edmond H. Vernot received his Bachelor of Arts in Chemistry from New York University in 1948 and did graduate work at Brooklyn Polytechnic Institute. His experience includes work in analytical research and methods development at American Cyanamid Corporation, Lederle Laboratories Division, and as Senior Chemist and Project Leader at General Foods Corporation, where his chief interests were instrumental analysis and physical chemistry. He is presently employed by Aerojet-General Corporation at Wright-Patterson Air Force Base and is responsible for a variety of analytical chemistry functions.

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